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No. 1.

THREE GLIOMATA OF EPENDYMAL ORIGIN: TWO IN THE FOURTH VENTRICLE, ONE SUBCUTANEOUS OVER THE COCCYX.¹

F. B. MALLORY, M.D.

(From the Sears Pathological Laboratory of the Harvard University Medical School.)

In 1890,² in announcing his discovery of a specific stain for neuroglia fibers, Weigert called attention to certain dots or granules (Körnchen) in the protoplasm of ependymal cells, which stain by the same differential methods that are used for neuroglia fibers. In his monograph "Die Neuroglia"³ he states that these granules are present not only in the ependymal cells lining the ventricles and the neural canal, but also, in adult cords, in certain cells and groups of cells which have been cut off from the neural canal by the neuroglia fibers and lie imbedded in them.

So far as known these granules do not occur in any other kind of cell; they afford, therefore, a very characteristic marking by which to distinguish ependymal cells from all other cells. Their significance is entirely unknown. Weigert speaks of them as "Körnchen" and "Punktierung," but does not describe them further. He has, however, proved definitely that they are not cilia.

Careful examination of these characteristic markings of ependymal cells shows that they vary considerably in size, shape, and number in different cells. They may be round, oval, or rod shaped; as the markings appear in the cells they strongly suggest minute cocci or delicate bacilli. The

¹ Read March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, at Cleveland, Ohio.

² Weigert. *Centralbl. f. allg. Path. u. Path. Anat.*, 1890, p. 736.

³ Weigert. *Die Neuroglia*. 1895.

rod forms may be from two to four times as long as broad; as a rule the ends are square. They vary from two to about ten in a clump in a single cell, while of the round form twenty to thirty are sometimes present. The phosphotungstic acid hematein method, which colors the protoplasm slightly, often shows them lying in a lighter staining area. They do not always adjoin the inner edge of an ependymal cell, but may lie at any point between it and the nucleus. The rod forms do not run in any one direction, but are irregularly clumped together.

As the following three gliomata present peculiarities which have not hitherto been described, it seems advisable to put them on record.

The first occurred in an adult and was pendent from the roof of the fourth ventricle. It was irregularly spherical in shape, measuring four by five by three centimeters, reddish gray in color, soft in consistence, and very vascular.

Microscopically the tumor consists of two very different structures, of cells which form a fairly coarse meshwork, and of large blood vessels, surrounded by connective tissue, which lie in the spaces in the meshwork. The relative proportions of cells and of blood vessels vary considerably in different parts, but on the whole the cells predominate. The cells are distinguished by two peculiarities: most of them contain in their protoplasm the markings characteristic of ependymal cells; and they have running between them and touching their protoplasm numerous delicate neuroglia fibers.

The cells are epithelial in type. The nuclei are of fair size, round to oval in shape, although sometimes irregular, and occasionally quite large. They contain numerous fine chromatin granules, but as a rule no distinct nucleoli. The protoplasm of the cells is fairly abundant in amount, although some of the cells possess little or apparently none. As a rule it is not distributed equally around the nucleus which in consequence often lies eccentric. The limits of the protoplasm are not well defined: it fades out at the periphery.

The cells show a marked tendency to form small clumps with fusion of their protoplasm. When this occurs the

nuclei are usually arranged at the periphery, but in no very definite order: the long axes of the nuclei may run in any direction. Only occasionally does one of these cell masses suggest the appearance of a giant cell.

In this mass of protoplasm there is often found a very small, round or irregular, sharply defined cavity like the lumen of a small gland. Occasionally as many as four of these cavities are present. Besides these apparent attempts at gland formation, many perfectly definite gland cavities are present in the tumor; the smaller ones are usually round, the larger, irregular. These cavities are lined by cells exactly like the ependymal cells. Like them they also contain the characteristic markings in the protoplasm on the side adjoining the lumen. Within some of the larger gland cavities a process similar to that seen in chronic ependymitis has occurred; that is, the ependymal cells are lacking in places and a mass of naked neuroglia fibers projects into the lumen.

The characteristic markings of the ependymal cells are found not only in the cells lining the gland cavities, as already mentioned, but also in the protoplasm of the clumps of cells and in many but not all of the single cells. In sections stained by the phosphotungstic acid hematein method, the markings are found to be almost invariably in a lighter staining area in the protoplasm. They show a tendency to arrange themselves around the periphery of this lighter area. They vary in number from about ten to thirty. In shape the markings vary from round and oval bodies to definite rods with square ends, which are from two to four times as long as broad. In the cell clumps there may be one large group of these peculiar bodies or as many small clumps as there are nuclei. When a minute gland-like cavity is present the markings are grouped in the protoplasm adjoining it.

The neuroglia fibers are not in any way remarkable; they are quite numerous, and fairly fine for the most part, although occasionally coarse; they run in all directions between the cells and cell-clumps.

The stroma of the tumor consists of very numerous, usually rather large blood vessels surrounded by a compara-

tively small amount of connective tissue. The walls of many of the blood vessels are hyaline. The remains of old hemorrhages are shown by areas of connective tissue containing numerous large pigmented cells.

In some parts of the tumor numerous corpora amylacea are present, always among the neuroglia cells from which in this case they seem to arise. One was found within the nucleus of a large neuroglia cell.

The floor of the fourth ventricle shows well-marked chronic ependymitis, and the cerebellar tissue adjoining the new growth considerable sclerosis. The remains of old hemorrhages and the absence of mitotic figures would seem to indicate that the tumor grew slowly.

The second tumor¹ likewise occurred in the fourth ventricle, which it completely filled. It formed a semitranslucent, grayish, somewhat pear-shaped mass measuring five by six and five-tenths by three centimeters. It was attached to the floor of the ventricle and the smaller conical end projected beneath the pia over the posterior surface of the pons and the beginning of the medulla. On section it presented the same grayish translucent appearance as the outer surface.

Microscopically the tumor is composed of areas consisting chiefly of neuroglia fibers and of other areas about equal in extent which are very cellular. The two forms of tissue are not very sharply marked off from each other, but tend to fuse together. The cellular areas closely resemble the appearance of the first tumor described. The cells tend to arrange themselves in clumps; occasionally there occur well-defined gland cavities lined with a definite epithelium. In this tumor as in the other small groups of the characteristic ependymal cell markings are present in the protoplasm of the cells. They are not so numerous as in the first case, but vary from two to about ten in number in a cell. As a rule they are round or oval, but occasionally rod-shaped. They are present in most of the cells in the cellular areas, in-

¹ I am indebted for this case to Dr. E. M. Holmes. Patient, a boy aged seventeen years; symptoms for over two years. Brain: convolutions flattened; interpeduncular space bulging; lateral ventricles much dilated; weight of brain after escape of fluid one thousand five hundred and forty grammes.

cluding the cells lining the gland-like cavities, and in many of the cells in the denser areas, but where the neuroglia fibers are most abundant, the cells are few in number, have practically no protoplasm around them, and no markings can be found.

Throughout the tumor the neuroglia fibers are all fine ; no coarse ones occur. Even in the cellular parts of the growth they are fairly abundant and run in all directions between the single cells and cell-clumps.

The finding of several mitotic figures is evidence that the tumor was growing rapidly. The blood vessels are not nearly so numerous as in the first case, are thin-walled, and are rather more numerous in the cellular parts.

The third tumor is much the most interesting of the series. It has always been taught that gliomata develop only in the central nervous system and in the eye. The following case, however, shows that they may occur elsewhere, and it is probable, now that attention is called to such a possibility, that other gliomata not directly connected with the central nervous system will be found.

The tumor in question was sent to me for diagnosis by Dr. Joseph M. Sheahan, of Quincy, Mass., to whom I am indebted for the following clinical history. The patient was a woman forty-four years old who had always enjoyed good health. The tumor had been present for twenty-five years to the patient's knowledge and was probably congenital, because when first noticed it was a nodule the size of a hickory nut situated in the median line of the back over the coccyx. It kept that form and size until about one year before removal, when it rapidly increased in size. The enlargement was particularly marked during the three months preceding the operation. At that time it had reached the size of a base-ball. It lay almost exactly in the median line over the coccyx and lower part of the sacrum. It was not attached to the underlying bony structures, and the skin covering it was freely movable. The operation was simple, consisting of an incision half around the base of the growth, and of enucleation with the finger. The wound closed by first intention.

A little over half the tumor was sent to me for examination. It consisted of a hemispherical mass partly covered with skin, and of several smaller pieces. The growth was dense, slightly lobulated, and definitely encapsulated. On section the surface was gray, translucent, and rather granular, with numerous irregular, opaque areas of necrosis scattered throughout it. In its gross appearance the tumor suggested a fibro-sarcoma. A small piece of the tissue was preserved in Zenker's fluid, and the rest was put on ice. The structure of the growth on microscopic examination suggested a glioma, and this diagnosis was confirmed later by differential stains.

When the nature of the tumor was suspected the tissue preserved on ice was immediately brought out and sections of it put into formaldehyde. As this was ten days after the operation, not much in the way of the usual differential stains of neuroglia fibers could be hoped for, although a low temperature has the property of preserving the peculiar chemical properties of neuroglia fibers remarkably well. Only fair results were obtained from this material. Fortunately that part of the tumor which had not originally been sent to me, had been carried off by an assistant at the operation and been by him preserved in formaldehyde. This tissue was later kindly placed at my disposal and from it a perfectly satisfactory differential staining of the neuroglia fibers was obtained.

Histologically the tumor closely resembles at first sight a carcinoma. It consists of a connective tissue stroma in the meshes of which lie masses of epithelial-like cells. In places the stroma is slight, the masses of cells large; in other places the stroma is very abundant and the tumor cells occur in small groups or in rows of single cells: in other words, the growth is partly medullary, partly scirrhus in type. On careful examination, however, the growth presents a very peculiar appearance due to the presence, between the cells in the alveoli, of comparatively coarse, homogeneous, refractive fibers which vary greatly in different parts of the growth, but in places are very abundant. For the most part the fibers in the alveoli tend to run in the same general direc-

tion. They are usually straight or a little curved, but occasionally are wavy or even corkscrew-like, as though confined within too narrow quarters. This latter appearance may, however, be an artifact.

It is impossible to determine the length of the fibers, but many of them certainly are long. One end of them seems always to start from the wall of an alveolus; the other end probably terminates in the same way. Just how the fibers end is difficult to decide. So far as can be made out they swell slightly, forming a sort of foot which ends squarely and stains less intensely than the rest of the fiber. The swollen ends unite laterally and thus form, at least in places, a fairly definite surface which is closely applied to the connective tissue stroma. In places the limiting surface thus formed has been stripped away from the stroma by the shrinkage due to the fixing reagent, and here its structure can be more readily studied.

The neuroglia fibers vary much in size. Some of them are very fine, but many of them are extremely coarse. A given fiber always preserves a uniform size; it does not branch, and does not begin or end in the protoplasm of a cell, although the fibers often touch the protoplasm in passing a cell. With the analin blue connective tissue stain the neuroglia fibers stain intensely red in marked contrast to the blue of the connective tissue fibrillæ.

The neuroglia cells in the alveoli are epithelial in type. The nuclei are vesicular, containing numerous fine chromatin granules, but no distinct nucleolus, and are usually oval in shape; occasionally quite large nuclei occur. The protoplasm around the nucleus is finely granular and usually fairly abundant; as a rule a process of protoplasm extends out on each side of the nucleus, running in the same direction as the neuroglia fibers; the limits of the cells are not sharply defined. The fact that the tumor is growing rapidly is shown by the presence of comparatively numerous mitotic figures which occur even in cells surrounded by numerous neuroglia fibers.

The connective tissue of the stroma is not remarkable. It

varies considerably in amount in different places, and occasionally sends small bundles of fibrillæ in between the neuroglia cells and fibers.

The interest in this tumor lies, of course, in its situation and in its histogenesis. The latter, it seems to me, admits of a simple explanation. In a paper on Sacro-Coccygeal Dimples, Sinuses, and Cysts,¹ published in 1892, I stated that examination of the tissues over the coccyx and sacrum of seven embryos showed in each of six of them one or more gland-like structures lined with epithelium. These I believed to be the remains of the lower end of the neural canal which closes very irregularly. It seems to me that the explanation of the tumor described in this paper is connected with these same embryonic remains. These cells unquestionably have, in consequence of their origin, the potential possibilities of differentiating either into cells like the epidermis (as seen in the dermoid sinuses and cysts found in this region), or into ependymal cells and their derivatives, in this case neuroglia tissue. This view is strengthened by the fact that in the more cellular parts of the tumor and to a less extent in the denser portions, from one to five minute rod-shaped bodies with square ends, and three to six times as long as broad, are present in the protoplasm of at least most of the cells. They stain by the differential methods for neuroglia fibers, but cannot be demonstrated in any other way. They may lie near the nucleus or at some distance from it in the cell process. They have no definite arrangement within the cell, although when the cell is elongated and the marking is single, its long axis usually coincides with that of the cell, but it may lie crosswise or oblique. By the phosphotungstic acid hematein method the markings are often seen to lie in a lighter staining area in the protoplasm. Twice they were found in cells in mitosis.

It would be interesting to study the fetal remains of the neural canal in embryos by means of the new differential stains to see if neuroglia fibers or the protoplasmic markings characteristic of ependymal cells are ever present; this I

¹ Mallory. Amer. J. Med. Sciences, 1892, Vol. 103, p. 263.

have not had an opportunity to do. I may add in this connection, however, that I have recently studied a case of spina bifida of the lower lumbar vertebræ below the termination of the cord, in a two-weeks-old infant, in which the lesion was due to an adenocystoma, of which the cells were lined with ciliated epithelium as are the cells lining the neural canal in early embryonic life. Unfortunately the tissue had not been fixed in such a way that it could be stained for the markings characteristic of ependymal cells.

Summary and Conclusions.

The first tumor is a very vascular slowly growing glioma of the fourth ventricle. The cells tend to fuse together into cell-clumps, some of which contain minute lumina. Definite gland cavities of various sizes are also present. The peculiar round, oval, and rod-shaped, differentially staining markings characteristic of ependymal cells are present not only in the cells lining the gland-like cavities, but also in the cell clumps and in many of the single cells. They vary from about ten to thirty in a group and are usually situated in the periphery of a lighter staining area in the protoplasm. Delicate neuroglia fibers are quite abundant throughout the tumor.

The second tumor, likewise from the fourth ventricle, is much less vascular, and is composed of cellular and dense areas of neuroglia tissue. In the cellular areas the cells tend to fuse together, and definite gland-like cavities occur. The markings peculiar to ependymal cells are present in the cells of this tumor also, but in smaller numbers than in the first. They vary from two to about ten in a cell.

The third tumor is interesting from its location, its structure, and its histogenesis. Situated subcutaneously over the coccyx, it resembles in its structure a carcinoma, consisting of masses of epithelioid cells embedded in the meshes of a connective tissue stroma; but between the epithelioid cells occurs a second intercellular substance, namely, fine and coarse fibers which stain by the differential methods for neuroglia fibers. Moreover many of the cells contain from

one to five minute rod-shaped markings which stain by the same methods.

In consequence of the presence of characteristic, differentially-staining markings in the cells of these gliomata, it seems reasonable to infer that the tumors are of ependymal origin. Possibly all gliomata of ependymal origin are definitely characterized in the same manner.

DESCRIPTION OF PLATE.

PLATE I.

(Figs. 1, 2, and 3 are from the first tumor described, and show the markings characteristic of ependymal cells.)

FIG. 1. — Cells in the wall of the bottom of a gland cavity.

FIG. 2. — A fused clump of cells.

FIG. 3. — A single cell.

(Figs. 4, 5, and 6 are from the glioma over the coccyx.)

FIG. 4. — Shows the neuroglia fibers terminating in swollen ends which unite laterally to form a sort of membrane which is applied to the connective tissue stroma.

FIG. 5. — Straight and corkscrew-like neuroglia fibers.

FIG. 6. — Cells showing single rod-shaped markings in the protoplasm. One is on end, the other four are lying flat.

THE PATHOLOGY OF A CASE OF POLIENCEPHALOMYELITIS.*

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Poliencephalitis was the name given first by Wernicke¹ to a spontaneous, acute hemorrhagic inflammation of the pons and medulla limited to the central gray matter of the third and fourth ventricles and the aqueduct of Sylvius. The disease according to Wernicke is one of adult life and the patients are usually alcoholics. The characteristic symptoms are sudden onset, progressive ophthalmoplegia externa, but usually without ptosis, somnolence, mental confusion, tottering gait, slight ataxia. The disease runs a rapid course and is fatal in ten to fourteen days. The anatomical changes according to Wernicke are found in the gray matter of the pons and medulla, and consist in numerous capillary hemorrhages, a serous and cellular exudate, and granule cells. He lays stress on the vascular changes, not on the degeneration of nerve cells, which indeed may not take place at all. He emphasizes the resemblance between this process and poliomyelitis, and says that the medulla and pons, as they form the connecting link between cord and brain, have the characteristics of both and may at one time show processes resembling those in the brain, as hemorrhage and softening, or again those in the cord as an independent inflammation of the gray matter. This latter process he calls poliiencephalitis hemorrhagica acuta and looks upon it as essentially the same as poliomyelitis, differing only in location. Later on, a distinction was made between two forms, — inferior and superior, — the superior involving the gray matter of the third ventricle and from this region down as far as and including the nucleus of the sixth, the inferior beginning below the nucleus of the sixth, and extending down to the pyramidal crossing.

Flatau² in his summary of the subject in 1900 adds two more subdivisions, the combination of the superior and in-

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ferior, and the combination of the two with involvement of the gray matter of the cord, or poli-encephalomyelitis. Following the description by Wernicke, a number of observers reported cases of poli-encephalitis superior, but in the great majority of these the diagnosis rested only on the clinical symptoms; either the cases never came to autopsy or the pathological findings were negative when an autopsy was made. Oppenheim³ in 1900 speaks of poli-encephalitis as a rare affection and one rarely accompanied by anatomical lesions demonstrable after death. He repeats the description given by Wernicke and agrees with him that the most important etiological factor is chronic alcoholism, a fact brought out in the earlier reports (Gayet, Wernicke, Thomsen), although later observers have found a disease clinically corresponding to Wernicke's description following the acute infectious diseases in persons with no alcoholic history. Oppenheim is not willing to accept all of these cases as poli-encephalitis, but admits that acute infection may be an etiological factor in the disease, and says that it is really an acute non-suppurative encephalitis with localization in the pons, cord, and medulla. He does not, therefore, emphasize the analogy between poli-encephalitis and poliomyelitis as Wernicke does and makes no clear distinction between this and acute bulbar myelitis.

This same confusion is found in Gower's⁴ treatment of the two diseases in the 1896 edition of his "Diseases of the Nervous System." He speaks of poli-encephalitis as a very rare affection of which few indisputable instances have been reported as yet. The description he gives is that of Wernicke, and he calls it an acute nuclear degeneration. In treating of acute bulbar myelitis, however, he gives much the same description and quotes as an example the case of Etter, in which there were degenerative changes confined to the nuclei of the motor cranial nerves. Gowers calls attention to the similarity between this process and poliomyelitis, and suggests the term "acute nuclear disease" for such a case, but never makes the connection between this and Wernicke's disease.

Church and Peterson⁵ refer but briefly to the disease. They give the usual division into poliencephalitis superior and inferior, and speak of the great rarity of the latter form especially. Von Leyden and Goldscheider,⁶ in Nothnagel's "Specielle Pathologie und Therapie," treat of the disease under the name of "Wernicke's poliencephalitis hæmorrhagica acuta superior" and give the symptomatology much as Wernicke did. There is here, however, the same apparent confusion between this disease and acute bulbar myelitis, or myelitis hæmorrhagica bulbi, as was noticed in Oppenheim's treatment of the subject. Under the latter head — myelitis hæmorrhagica bulbi — the authors describe an acute inflammatory process in the medulla involving both gray and white matter. Von Leyden's case is given as a type of this form. He found multiple foci of softening and hemorrhages which were most numerous in the region of the olives, and involved especially the pyramidal tracts and inter-olivary region. The gray matter was involved, but the most prominent changes were in the white. This the authors call "Myelitis bulbi hæmorrhagica." Under the head of poliencephalitis superior (Wernicke) a process essentially the same as this is described, and the only distinction between a bulbar myelitis and a poliencephalitis is lost when the authors state that the changes in poliencephalitis, although predominant in the gray matter, are frequently found also in the white, and not secondarily to the degeneration of the gray, for the intramedullary nerve tracts are more apt to be degenerated than are the cells of the nuclei. And the two instances cited under this head, Goldscheider's case and that of Strümpell, were both primary inflammations of white tracts as well as gray matter. It is indeed hard to see why Von Leyden's case and Goldscheider's case should be classed as two different processes. The distinction between myelitis as a diffuse, disseminated inflammatory process, and poliomyelitis as an inflammation confined to the gray matter, a distinction clearly established in the case of the cord, is apparently ignored when the process is higher up. That a secondary degeneration of the white tracts will always occur in cases of

long duration when the cells of the nucleus are degenerated was pointed out by Kaiser.⁷ The disease of Wernicke is primary in the gray matter, which always shows the inflammatory changes first, and in many cases death occurs before the disease has gone any farther; but if it is of longer duration the white tracts also become involved secondarily.

Even if we include in the list the cases where the anatomical changes seem to have been those of acute bulbar myelitis rather than those of Wernicke's disease the number is still very small. Gayet's⁸ case is perhaps the first indisputable one, Wernicke himself citing it as such. Gayet called it "encéphalite diffuse." The case was one of much longer duration than is usual, according to Wernicke, but the anatomical changes were typical: inflammation and capillary hemorrhages in the central gray matter of the third and fourth ventricles extending from the anterior commissure to the end of the calamus scriptorius and involving both thalami; the nerve cells, however, were not degenerated. The patient was an adult alcoholic.

Wernicke's three cases were all adults, two alcoholics, the third a case of sulphuric acid poisoning. In all there were punctiform hemorrhages in the gray matter of the third ventricle, aqueduct, and fourth ventricle, without any degenerative changes in the blood-vessels. No degeneration of the nerve-cells was found, and the white matter was not involved. Thomsen's⁹ three cases seem also to belong to this type, although in all these cases there was a peripheral neuritis as well as central lesions. The first was an adult alcoholic, and the duration of the disease was twelve days. Multiple peripheral neuritis was found and an inflammation with hemorrhage involving the nucleus of the hypoglossal, the dorsal nucleus of the auditory, the nucleus of the pathetic and the motor oculi. There was, however, no degeneration of the ganglion cells in these nuclei. His second case was of longer duration, twenty days. In this medulla the hemorrhages were not so numerous as in the first one, but degenerative changes were found in the nerve cells of the nuclei affected, — the nuclei of the hypoglossal, abducens,

patheticus, and motor oculi. There were also sclerotic patches in the blood-vessels. The third case resembled the first in the extensive hemorrhages and absence of degeneration in the nerve cells.

The case of Wijnhoff and Scheffer¹⁰ I have been able to find only in Goldscheider's article, in which a short abstract of the pathological changes is given. The man was an alcoholic, sixty-two years of age. After death multiple capillary hemorrhages were found around the aqueduct at the level of the anterior corpus quadrigeminum, shrinking of the ganglion cells, disappearance of the Nissl bodies, and in some cells loss of nucleus; increase of neuroglia cells and of lymphoid cells. The same condition was found in the floor of the fourth ventricle. No mention is made of the exact distribution of these changes.*

Kaiser's⁷ case is quoted by Oppenheim as a case of Wernicke's disease with more extensive lesions than any others yet reported. The author himself calls it "poliencephalitis superior acuta, Wernicke," in spite of the long duration—seven weeks—and the involvement of the white matter. There were no macroscopic changes except tiny foci of inflammation extensively scattered throughout cord and medulla in the white matter as well as in the gray. The vessels within these foci were dilated, the perivascular spaces filled with cells which, in the case of the capillaries, had also wandered into the surrounding tissues. The cells were rather large and with pale nuclei; granule cells were not found. Small hemorrhages were also seen. There was degeneration of the nuclei of the twelfth, tenth, and ninth on both sides, of the nucleus ambiguus on the left, the entire dorsal nucleus of the eighth on the left and part of it on the right, the nucleus of the seventh on the left, both nuclei of the sixth, the motor and sensory nuclei of the fifth on the left, and the nucleus of the fourth and of the third on both sides; also of the cells of the anterior horns in the cervical

* Kojewnikoff (Le Progrès Médical, 1887) reports a case of poliencephalitis superior in an adult alcoholic. The lesions found were symmetrical, consisting in congestion of the vessels and capillary hemorrhages in both thalami and in the central gray matter of the pons, involving the oculo-motor nucleus.

enlargement. The fibers of the twelfth nerve on both sides were degenerated more than the nuclei of these nerves; the roots of the fifth, both ascending and descending, the fibers of the eighth and ninth, the knee of the seventh and the fibers of the fourth and part of the third on the left side, and the fasciculus longitudinalis posterior on that same side. As the cord was involved, Kaiser suggests the name of poli-encephalomyelitis for this condition.

Among the cases which in most respects correspond to Wernicke's description, but show little or no hemorrhage, we may count those of Etter,¹¹ of Rissler,¹² and of Kalischer.¹³ In Etter's case the medulla was normal to the naked eye, but microscopic examination showed foci of inflammation in the gray matter involving the sixth nucleus on both sides, the seventh nucleus of one side, and the fibers on the other, the motor nucleus of the tenth, and the nucleus of the twelfth on both sides. Etter's case is the one quoted by Gowers as acute bulbar paralysis. Rissler's case, quoted by Goldscheider, showed vascular changes, but only slight hemorrhages. There were large numbers of mononuclear elements in the perivascular spaces and also in the tissues. In some places there were changes in the ganglion cells, swelling, loss of processes, paleness of the nuclei, but these changes were not found in places where the vascular infiltration was greatest, so that there was no direct relation between the changes in the vessels and those in the nerve cells.

The case reported by Kalischer is by him regarded as a combination of primary atrophy and of poli-encephalitis hemorrhagica acuta superior, but Leyden and Goldscheider class it as a not altogether typical case of asthenic bulbar paralysis with anatomical lesions. Kalischer's description is as follows: Patient sixty-four years old, non-alcoholic. Duration of disease four months and a half. There were found after death degeneration of the cells of the third nucleus, tiny hemorrhages near the tenth nucleus, the sixth nucleus and elsewhere, with a great dilatation of the vessels, but without cellular infiltration of the tissues. This process he regards as poli-encephalitis superior acuta hemorrhagica; but in the

nucleus of the fourth and sixth nerves and in the anterior horns of the cervical cord he found simply shrinking of the ganglion cells and diminution in their number, while the vascular changes here were very slight, and he interprets this process as a primary atrophy. The case, therefore, is not typical, either of the acute inflammatory or of the subacute atrophic form. Yet it is difficult to see why it should be classed as asthenic bulbar paralysis.

Two cases, those of Oppenheim³ and Patrick,¹⁴ showed such very slight lesions as to lead the investigators to doubt whether they had to do with an acute inflammation of the type of Wernicke, or simply a neurosis. In Patrick's case the clinical symptoms pointed to acute degeneration of the nuclei of the external eye muscles, of the nucleus of the seventh, of the ninth, and tenth, but after death the only changes found were engorgement of the blood vessels without hemorrhage or migration of cells, and a slight degeneration of the cells of the sixth and seventh and of some of the intramedullary fibers of the seventh. Patrick considered this case as lying between poliiencephalitis and asthenic bulbar paralysis, and the same opinion is held by Oppenheim, who quotes Patrick's case and gives one of his own. The symptoms were the same as in Patrick's. The post-mortem findings consisted in slight hemorrhages into the gray matter of the crura and cervical cord, with slight round cell infiltration, hyaline degeneration of the blood vessels, and a linear scar in the cervical enlargement.

The cases in the literature usually classed as poliiencephalitis (Wernicke) which seem, strictly speaking, to correspond more to the diffuse inflammatory process, to acute bulbar myelitis, are those reported by Von Leyden,¹⁵ by Eisenlohr,¹⁶ by Strümpell, and by Goldscheider.¹⁷ Eisenlohr found, in a patient dying with symptoms of Wernicke's disease, multiple hemorrhages scattered in the gray and white matter of the crura, corpora quadrigemina, thalami, and pons; a peripheral neuritis existed here also. Goldscheider's case is also, strictly speaking, a myelitis rather than a poliiencephalitis, as the white matter is extensively involved. Large areas of de-

generation with hemorrhages and infiltration of round cells were found in the pons and crura involving the central gray matter, the lemniscus, brachium conjunctivum, substantia nigra, pes pedunculi, red nucleus, thalamus, posterior commissure, internal capsule, and centrum ovale. The cells he considers not emigrated leucocytes, but proliferated cells from the endothelial lining of the lymph spaces and capillaries, also proliferated neuroglia cells.

Strümpell's case is given by v. Leyden and Goldscheider as an instance of Wernicke's disease; inflammatory edema, and hemorrhage in the central gray matter of medulla and pons, in the cortex, in the centrum ovale, corpus striatum, and thalamus. There was no softening, no degeneration of the nerve cells, no granule cells.

These cases are all adults, and in most of them chronic alcoholism is the most important etiological factor. The term "acute" applies evidently to only a few. According to Leyden and Goldscheider, those of long duration are apt to show greater changes in the nerve cells, while Thomsen, Gayet, and Kaiser think that there is no degeneration at all of the nerve cells in the rapid forms, only vascular engorgement and exudation; the cells are found degenerated in the subacute and chronic forms. Thomsen found degenerated cells in his twenty days' case, but not in his twelve days'.

Rissler, however, finds no relation between the intensity of the vascular engorgement and exudation and the changes in the cells, but believes on the contrary that there is a primary atrophy of the nerve cells quite in the sense of Charcot, in which opinion Kalischer seems to concur. A totally different view from that of Wernicke is held by Thomsen⁹ and Jacobaeus,¹⁰ namely, that the cases of poli-encephalitis, so-called, in adult alcoholics are in reality cases of alcoholic polyneuritis with accompanying changes in the medulla. Thomsen's cases have been already quoted, and Eisenlohr's belongs here also. That of Jacobaeus was an alcoholic with multiple neuritis, and punctiform hemorrhages were found in the gray matter from the third ventricle to the ala cinerea, but Jacobaeus thinks, as does Thomsen, that these changes

in the medulla are simply a complication of the polyneuritis, just as a myelitis of the cord may complicate it. This is, however, too sweeping an assertion. The cases of these two authors prove that poliencephalitis and polyneuritis may be coincident, and it is possible that other cases of poliencephalitis might have been shown to be accompanied by inflammation of the peripheral nerves if an examination of the nerves had been made; but it is certainly undeniable that poliencephalitis exists without any involvement of the peripheral nerves.

Short as is the list of cases I have been able to collect from the literature, it is yet longer than that given by most authors who treat of the subject. Oppenheim⁸ in an article in 1899 accepts only the cases of Kaiser, of Kalischer, and of Marie (which I have not been able to find) and, as doubtful cases, those of Patrick and of Oppenheim. It seems therefore worth while to add to the literature another case, which, although differing from all those yet reported, in the youth of the patient and in the rapidity of the course of the disease, is yet undoubtedly a case of Wernicke's poliencephalomyelitis. The case was one which occurred in the practice of Dr. Hugh T. Patrick, to whom I am indebted for the following clinical history:

Elizabeth C., a child of five and a half years, was first seen by Dr. Patrick in consultation on Tuesday evening. She had been perfectly well up to the previous Friday, when she seemed somewhat indisposed and was given a dose of castor oil. This moved the bowels freely the following day and after that she seemed quite well. During Sunday she was lively, active, and apparently well in every way. Sunday evening she had a very tearful parting from an old nurse; on Monday she seemed unwell and vomited a number of times. She was seen by the family physician, who thought her not seriously ill, did not take temperature or make a thorough examination. When he saw her again on Tuesday the vomiting had continued, she had eaten nothing, had a temperature of 102° F., protruded the tongue in an irregular and spasmodic fashion; he noticed some irregularity in volitional movements of the right hand and noticed also a difference in the two sides of the face when she cried or vomited.

When seen by Dr. Patrick in the evening of the same day she had practically complete paralysis of the right side of face, the eye remaining wide open when she cried and the brow not wrinkling. There was decided incoördination of the right hand and apparently slight incoördina-

tion of the left; pupils equal and normal; very slight nystagmus on lateral movement; tongue protruded straight; hearing on the right side better than on the left; when placed on her feet she walked perfectly well. She had scarcely spoken during the day except a monosyllable now and then. She had vomited about eighteen times during the day, was restless, irritable, peevish. Apparently she understood everything that was said to her and complied with simple requests. Dr. Patrick made the diagnosis of acute nuclear disease.

At four o'clock the following morning the pulse was one hundred and thirty, temperature 102.8° F., respirations forty-eight to fifty and somewhat irregular, approaching in type the Cheyne-Stokes. There was weakness of the right internal rectus, increased incoördination of the right hand, increased mental hebetude. There had been no more vomiting. She was sponged and afterwards slept for nearly an hour. Was more quiet, with considerable hebetude, and it was impossible to get her to say a single word, although she seemed to recognize people. Death occurred suddenly on Wednesday afternoon.

I have no report of the autopsy except that the findings were negative. The brain and medulla were given me for examination, but unfortunately the cord was not removed, so that my investigations extend down as far as the lower end of the pyramidal decussation only. The material, which had been hardened in formalin, was embedded in celloidin, and serial sections were cut from the lower end of the pyramidal decussation to the level of the anterior corpus quadrigeminum. Sections were stained by Nissl's method, by Van Gieson's method, and by the usual methods for demonstrating cell structure; hematoxylin and eosin, iron-lack hematoxylin and erythrosin, Upson's carmine, methylene-blue, etc., etc. Marchi's method was not tried, the duration of the disease being so short as to render it highly improbable that any degeneration would be demonstrable by this method. Chiefly for the purposes of orientation five out of every twenty sections were stained by Weigert's method.

In the cervical cord at the beginning of the decussation the changes found were those typical of an acute poliomyelitis; the vessels of the gray matter were engorged, the perivascular spaces filled with round cells which were largely mononuclear. In the gray matter of the anterior horns and occasionally in the posterior, these cells were found not only around the vessels but in the tissue, sometimes diffusely

scattered, sometimes in tiny groups. These cells were almost all mononuclear, some of them small and round with round, deeply-staining nucleus, others large, with an oval, vesicular nucleus. A few of them were multinuclear, and occasionally polymorphonuclear leucocytes were seen, but usually only in the collections around vessels. There were no granule cells, nor were the fine medullated fibers of the gray matter much altered. Only a few swollen nerve fibers and axis cylinders were found. On the other hand the changes in the multipolar nerve cells of the anterior horns were very pronounced, especially on the right side. On the left side two or three normal cells could be found in each section (the sections were ten μ thick), but on the right never more than one and not always even one normal cell was found. The cells had undergone one of two forms of degeneration: either swelling with central chromatolysis, the Nissl bodies showing only as granules around the edge, the nucleus often pushed to one side and diffusely stained, the protoplasmic processes swollen; or a sclerotic change, the cell body shrunken and deeply stained, the nucleus shrunken, staining diffusely, the processes atrophied. The vessels of the pia, especially those in the antero-median fissure, were distended, but there was no perivascular infiltration of the extra-medullary vessels.

Between the upper end of the decussation and the exit of the hypoglossal nerve the changes in the cord were much less extensive than below. The cells of the nuclei, including the anterior nuclei, were normal and the cellular infiltration was seen chiefly around the vessels and capillaries, but beginning at the exit of the twelfth nerve the changes became gradually more severe. The gray matter of the fourth ventricle and the substantia reticularis grisea were filled with round cells in tiny groups and with numbers of scattered epithelioid cells, whose nuclei showed the effects of active ameboid movements. Very few of these cells were polymorphonuclear leucocytes. A decided difference could now be seen between the two sides; the amount of cellular infiltration was much greater on the right side. The gray matter

was always the seat of these changes; the white tracts were unaffected except for the distention of the blood-vessels. As was the case in the cord, the meningeal vessels showed no perivascular infiltration and nowhere was thrombosis seen. The nerve cells of the hypoglossal nucleus stained by Nissl's method were normal, so were the fine nerve fibers in this nucleus, yet tiny foci of cellular infiltration were found here, especially on the right side. The same is true of the nucleus *alæ cinereæ*, the *substantia gelatinosa*, and the nucleus *ambiguus*, and the dorsal nucleus of the eighth nerve. Weigert sections showed no degeneration of the nerve fibers.

At the level of the upper end of the olive the changes described were all intensified. The perivascular infiltration of the vessels of the gray matter was greater, and the number of wandering cells in the gray matter was increased, the difference between the two sides more marked than ever. On the right side the nucleus of the facial nerve was found to be much damaged. It stood out as a light area devoid of medullated fibers, in Weigert sections, and in Nissl's sections showed large numbers of epithelioid, polymorphonuclear, and small mononuclear cells with degenerated nerve cells. The actual number of the nerve cells seemed to be decreased and, as most of them were much shrunken, and many very pale, the contrast between this nucleus and the normal one on the left was very striking, especially in hematoxylin and Nissl's sections. In the Nissl sections various forms of degeneration were found in these nerve cells. Perhaps the most common was a shrinking of the cell body, but not a marked distortion of the shape, disappearance of the Nissl bodies from the dendrites and often from the periphery of the cell body, a deeply stained central zone which sometimes seemed granular, but more often had a "honey-combed" appearance, diffuse staining of the nucleus so that it was often impossible to distinguish its outline. (Plate II., Fig. 1.) The nucleolus in these cells was unchanged. Other cells not so numerous showed a central instead of a peripheral chromatolysis, the stainable

substances collecting in granules on the edge of the cell, leaving the center pale. The nucleus was apt to be at one side of the cell, the chromatin diffusely staining, the nucleolus normal. There were also very much atrophied cells, some with shrunken dendrites, some with none at all. These shrunken cells stained deeply, and the nucleus was usually indistinguishable although the nucleolus was always visible. Very decided distortions of shape were seen in these cells. A fourth variety consisted of very pale cells from which the stainable substance had completely vanished; usually the nucleus could be seen, distorted and pale, sometimes with, sometimes without a nucleolus. (Plate II., Fig. 2.) The degeneration of this nucleus extended throughout the lower half of its extent, then the normal nerve cells began to reappear, the degenerated ones became fewer, and finally the very uppermost end of the nucleus seemed perfectly normal except for the foci of wandering cells.

The same changes, but less intense and of less extent, were found in the nucleus of the sixth nerve on the same side, disappearance of some of the nerve cells, degenerative changes in those which remained, infiltration with wandering cells, swelling or breaking down of the fine medullated fibers in the nucleus; but the portion of the nucleus thus damaged included only about one-third of its extent. The changes disappeared more rapidly than in the nucleus of the seventh, and the upper half of this nucleus was perfectly normal. The condition described was strictly unilateral, both nuclei on the left side were unchanged. Nor could any degeneration be detected in the intramedullary roots of the sixth and seventh nerves on the injured side; the substantia gelatinosa, the nuclei and roots of the eighth were also unchanged. From this point on, infiltration of the tissues was less extensive although there were still foci of wandering cells, and the perivascular infiltration diminished very slowly, persisting longest in the vessels of the central gray matter and on the right side. The cells of the motor nucleus of the fifth nerve, of the substantia ferruginea, and of the accessory motor nucleus of the fifth were normal.

At the level of the lower end of the nucleus of the fourth the appearance was altogether different from that at the level of the seventh. The gray matter and white matter of the tegmentum were full of hyaline bodies, in many places the tissue was rarefied as if from edema, while the migration of cells was not nearly so extensive, and there was no sign of degeneration in the nerve cells. These hyaline bodies appeared quite suddenly at the level of the lower end of the fourth nucleus and persisted in large numbers as far up as the beginning of the exit of the third nerve; then the numbers began to diminish, but a few were still found throughout the red nucleus.

The nuclei of the fourth and third nerves were normal, as far as the nerve cells and fine fibers were concerned, but they showed some of the cellular infiltration universal throughout the gray matter. From this point up, the only changes found were the hyaline bodies already mentioned, distention of the blood vessels, and slight perivascular infiltration and edema, the two sides being now alike. The thalami on both sides were normal. Numerous sections from different areas of the cortex showed only vascular changes, the vessels were engorged and the perivascular tissue edematous, but there was no cellular infiltration, no hyaline bodies, the pyramidal cells and the nerve fibers absolutely normal.

To Recapitulate. — Engorgement of the vessels of the gray and white matter throughout cervical cord, medulla, pons, and crura. Perivascular infiltration of round and oval cells in the gray matter and to a less extent in the white matter from the lower end of the decussation of the pyramidal tracts to the exit of the third nerve, this infiltration more marked on the right than on the left. Degeneration of the multipolar cells of the anterior horn of the cervical cord especially on the right side, of the cells of the lower two-thirds of the nucleus of the seventh nerve on the right, and of the lower third of the nucleus of the sixth. Edema and numerous hyaline bodies in the gray and white matter and in the perivascular spaces in the tegmentum of the pons at the level of

the nucleus of the fourth nerve extending beyond the exit of the third nerve. Engorgement of vessels and edema in brain. No capillary hemorrhages, no degeneration of the white tracts.

That this is a case of primary acute inflammation, toxic in origin, of the gray matter of the cervical cord, medulla, and pons seems most probable, although it differs from the typical poliencephalitis acuta hemorrhagica of Wernicke in several respects. Wernicke's disease attacks adults, usually alcoholics; this patient was a child of five. The nuclei involved usually form themselves into two groups, those of the external ocular muscles, third, fourth, and sixth — poli-encephalitis superior; or the motor nuclei between and including the seventh and twelfth — poli-encephalitis inferior. These forms may be combined wholly or in part, but so far as I have been able to discover, the involvement of the gray matter of the cervical cord with the nucleus of the sixth and of the seventh has never been described, though a combination of the cervical cord, the twelfth nucleus and the seventh, is not so uncommon. Wernicke describes the disease as acute, but the duration as given by him — seven to fourteen days — is far longer than in this case, which ran an unusually rapid course, being fatal in less than three days.

Typically the poli-encephalitis of Wernicke is a hemorrhagic inflammation, the capillary hemorrhages visible to the naked eye being the principal lesion; but as we have already seen, several cases have been described in which no hemorrhages were found, and this only adds another to the list. Much more unusual than the absence of hemorrhages is the rapid degeneration of the nerve cells. I can find no case of such short duration reported in which degeneration was found in the nerve cells — indeed, as I have already pointed out, most authors are agreed in regarding the vascular dilatation and exudation not only as the primary but as the sole changes in cases rapidly fatal, and Thomsen explains the absence of cellular degeneration in his twelve-day case by the short duration of the disease. In general it is certainly true that

changes in the nerve cells are found in the subacute and chronic forms, but not in the acute.

The remarkable harmony between the clinical history and the pathological findings in this case deserve to be pointed out in view of the great disparity between the two so often found, yet the harmony is not complete even here. The respiratory symptoms would lead us to expect degenerative changes in the nuclei of the tenth, but these were found to be normal. The respiratory disturbances, however, came on much later than the paralysis of the sixth and seventh nerves, and we know that the earliest stages of degeneration in nerve cells are not demonstrable by our staining methods.

The exact nature of the wandering cells deserves some mention. In the spaces of the adventitia these cells were of four kinds; the most numerous were the small mononuclear with round, deeply-stained nucleus; next, and nearly equaling them in number, were the large pale epithelioid cells, usually showing the changes due to ameboid movements and evidently arising by proliferation of the endothelial cells of the perivascular lymph spaces. Polymorphonuclear leucocytes varied in number, but were never very conspicuous, and finally there were a few small giant cells with never more than four nuclei. No plasma cells were found. Out in the tissue the small mononuclear cells were found composing the tiny clumps so often seen, while the diffuse infiltration was due to the migration of the epithelioid cells. There seemed to me no evidence of proliferation of the neuroglia.

The hyaline bodies are interesting from several points of view. I have purposely refrained from calling them amylaceous, or colloid bodies, for they do not correspond in their staining properties with either of these substances. They give only one of the specific amyloid reactions (reddish violet color with methyl violet), but do not turn blue with iodine and sulphuric acid, do not stain with osmic acid; stain in the same way as the surrounding tissues with Lugol's solution, and with methyl green; with hematoxylin and eosin they stain blue, with Van Gieson's stain pale blue — distin-

guishing them from the so-called Russell bodies. On the other hand, they do stain in the same way as does mucin, deep blue with methylene-blue, orange-yellow with safranin, reddish-violet with thionin or toluidin blue. A further distinguishing point between these bodies and corpora amylacea is the absence of concentric striations or radial striations. The bodies are irregularly roundish or oval; they look more like drops of candle grease than anything else as far as shape is concerned. Their size varies from that of a neuroglia cell to thirty-five mikrons. Usually they lie free in the neuroglia, the meshes of which seem pushed apart. Again they are found in the perivascular tissue, in the ventricle, and finally within the smaller vessels, where they often partially fill the lumen, less often completely fill it. Thus they differ in chemical nature, in morphology, and in distribution from the real amylaceous bodies. It is true that in the first two ways they correspond to the so-called amylaceous bodies of the central nervous system described by Redlich.¹⁸ This investigator examined the amylaceous bodies in the central nervous system of adults and young people dying of various diseases (not alone diseases of the nervous system), and arrived at results quite different from those of others, as Virchow,²⁰ Friedreich,²¹ Posner,²² Siegert,²³ Lubarsch and Ostertag.²⁴ He does not find concentric striations in these bodies, nor find them giving the amyloid reactions; according to his description the bodies stain exactly as do those in my case, with the exception of the stains for mucin, which he does not mention. Lubarsch and Ostertag refuse to consider them amylaceous bodies. They follow the classification given by Siegert, which distinguishes between true corpora amylacea and corpora flava; the former are concentrically striated, stain deep brown with Lugol's solution, blue with iodine and sulphuric acid, and give the characteristic amyloid colors with the aniline stains; the latter are never striated, are more irregular in outline, stain yellow with Lugol's, and give none of the amyloid reactions. These are the ones described by Redlich; they are sometimes found in the central nervous system according to Siegert; according to Redlich they are

the ones normally present there after middle life. The corpora amylacea of the central nervous system described by Virchow gave the amyloid reactions.

In spite, however, of the apparent correspondence between the bodies in my case and those described by Redlich, it is hardly probable that they are really the same.

The distribution of the hyaline bodies in my case is quite different from the distribution of the amylaceous bodies in Redlich's cases. Redlich found them but once in a case under thirty years of age; he looked upon them as normal after middle life, and thought that pathological processes had nothing to do with their formation except to increase the number. Their position in the perivascular tissue and within the blood vessels in my case suggests that they are formed by the coagulation of an exudate. Although this is not in accordance with the view usually held as to the formation of amylaceous bodies, yet Friedreich gave this explanation for the bodies found by him in a lung infarct, and Hayem²⁸ mentions finding colloid masses in the perivascular spaces and in the ventricle in two cases of poliomyelitis. Virchow, Ceci, and Rokitansky think them formed from the myelin of degenerated nerve fibers; Siegert, that they are true concretions, a deposit around a nucleus of degenerated cells. Lubarsch and Ostertag think that some are formed by mast cells and wandering cells. Redlich thinks that they are products of the nuclei of the cells of the neuroglia; Klein, that they are formed from Altmann's granules. By most authors the statement is made that corpora amylacea and corpora flava are found wherever neuroglia is proliferating at the expense of nerve tissue. In the case in question, however, there is no degeneration of the white fibers, no proliferation nor degeneration of the neuroglia, and in the regions where there is an actual breaking down of the nerve cells, no hyaline bodies are found. The tissues in the regions where the hyaline bodies lie does not show nearly as severe inflammatory changes as are found elsewhere, but it does show edema. In short, the evidence seems to point to the formation of these bodies by the coagulation of an exudate,

but in all probability this coagulation did not occur during life, for there is no sign of the changes which would necessarily be present, had these bodies formed within the blood vessels while the circulation was still active. There is no trace of infarction. Whether the coagulation occurred as a post-mortem change or as a result of the hardening fluids is impossible to say, but it could not have occurred during life. Yet there must have been a difference in the chemical nature of the exudate in this part of the pons, for although rarefaction from edema is found elsewhere, especially in the brain, the hyaline bodies are found only at the levels of the third and fourth nuclei.

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DESCRIPTION OF PLATE II.

FIG. I. — From the nucleus of the seventh nerve on the right, showing nerve cells with peripheral chromatolysis; one sclerotic nerve cell in lower left corner. Infiltration with epithelioid cells.

(Nissl stain: Leitz $\frac{1}{2}$ homog. immers. ocular iii.)

FIG. II. — Degenerated nerve cells from the right seventh nucleus; two normal cells from the left seventh nucleus.

(Nissl stain, slightly enlarged from Leitz $\frac{1}{2}$ homog. immersion, ocular iii.)

FIG. III. — Small vessel from the gray matter near the floor of the ventricle at the level of the seventh nucleus, showing perivascular infiltration. Large epithelioid cells seem to be derived from the endothelium.

(Hematoxylin stain: Leitz $\frac{1}{2}$ homog. immers. ocular iii.)

FIG. IV. — From the gray matter of the tegmentum at the level of the fourth nucleus, showing hyaline bodies; at (a) is shown a capillary vessel occluded by a hyaline body; at (b) one partially occluded.

(Toluidin stain: Leitz obj. $\frac{1}{2}$, ocular iii.)

A TUMOR-LIKE LESION IN THE LUNG OF A HORSE CAUSED
BY A BLASTOMYCES (TORULA).*

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The literature of blastomycetic infection has been so thoroughly quoted by recent writers, especially Ricketts¹ and Nichols,² that I refer those interested to these authors, and will mention only two works here since they deal especially with blastomycetic lesions in animals.

Fermi and Aruch³ describe a pseudo-glanders in the horse known as "Linfangite epizootica," "Farcin de rivière," or "Farcin d'Afrique." The disease closely resembles glanders and farcy with the exception that the lungs are practically never involved. From the lesions a blastomyces was obtained which grew readily upon potato, the colonies at the end of three days being elevated and dirty white with a smooth non-glistening surface. On agar, gelatine, etc., there was an exceedingly scanty growth. Horses, rabbits, and guinea-pigs were inoculated with this organism with negative results, unless we except rabbits which were inoculated in the testicle with pure cultures, after they had received an injection of ten cubic centimeters of one per cent lactic acid and thirty per cent grape sugar three times a week for three weeks; after inoculation they again received injections of the above solution for two weeks longer, and showed suppuration in the testicle and between the abdominal muscles. An unsatisfactory illustration of the specific organism leaves its classification in doubt.

Tokishige⁴ describes a pseudo-glanders occurring in horses and cattle in Japan which he says is identical with "Lymphangitis epizootica." The skin lesions are the more

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common and are sharply defined nodules from a pea to a walnut in size, which may remain hard, but usually suppurate and ulcerate. The lymph nodes near infected regions are generally involved. There seems to be a predilection for the testicle, where the process begins on the scrotum and prepuce, extending thence to the organ itself. The focus in the testicle is defined and resembles a tumor. Glanders-like lesions are often seen upon the nasal mucous membrane and may extend from here to the pharynx, larynx, and trachea; they are seldom seen in the larger bronchi and never in the small, yet the lungs may rarely be involved. (The lung lesions are not described.) In all diseased parts Tokishige found blastomycetes either free or in leucocytes (from his plates I should question the character of the including cells). He obtained pure cultures of this organism upon different media. The growth upon agar was hardly visible before thirty days, and after forty to fifty days the colonies were one to four millimeters in diameter, white and compact; it was difficult to remove a small portion with the needle and to crush it under the cover glass. In gelatine, colonies appeared after fifty-six days. Upon potato the growth was more rapid and of a light brown color (the time is not mentioned). Tokishige made many inoculation experiments, using a number of guinea-pigs, rabbits, dogs, cats, calves, horses, and swine. Pure cultures were used in some of these experiments, and in others direct inoculation from diseased portions such as the contents of ulcers, nasal secretions, and portions of nodules from the testicle, was attempted. Inoculations were made in a variety of ways and feeding experiments were included, but the results were negative. His illustrations show that the organism which he isolated forms a mycelium.

GROSS LESION. — The lesion which prompts this communication and the organism causing it differ essentially from those just described, and as far as I have examined the literature nothing analogous has been recorded. This supposed tumor was situated in the posterior portion of the caudal lobe

of the right lung of a horse, and was about as large again as the human head. A cut made through this large nodule showed its central portion to consist of a fairly firm gelatinous mass of a delicate pinkish-yellow color in its living portions, and containing large and small areas of necrosis. It so closely resembled a myxosarcoma that several pathologists unhesitatingly made this macroscopic diagnosis. This central portion was here and there loosely attached to a dense connective tissue wall, in other places separated from it — sequestrum-like — by a thick, yellowish, and sometimes reddish mucoid material not unlike sputum. Removal of the central mass left a cavity, to the wall of which still adhered portions of the central part of the growth. This wall consisted of dense connective tissue varying in thickness from six to less than one centimeter. Posteriorly this thick capsule gradually gave way to more or less normal lung tissue in which small white foci were distinctly visible, and which resembled miliary tubercles or areas of chronic broncho-pneumonia. (Microscopic examination of these foci gave the typical picture of chronic broncho-pneumonia; no blastomycetes were associated with the process.) In the thick wall of the lesion were a number of openings, the largest about four centimeters in diameter, which contained more or less of the sputum-like material above referred to. These communicated with each other and probably with the lung beyond, and it is assumed that they were dilated bronchi, though the specimen was so mutilated when it reached me that its true relationships could not be studied. That they undoubtedly were connected with the bronchi is borne out by the clinical history of the animal; for a year before he was killed an ever-increasing discharge from the nostrils was observed, particularly diffuse during exercise. For this reason he was suspected of having glanders, and twice, a few months previous to his death, guinea-pigs were inoculated in the usual manner for diagnosing glanders, the swab having been taken from this horse's nose. Both tests resulted in the death of the guinea-pigs from peritonitis. Whether there were metastases in the kidneys or other organs of this horse

is unknown, as only the lungs were examined by the person making the autopsy.

HISTOLOGY. — A microscopic examination of the central or myxomatous-like portion of the lesion showed it to consist of delicate trabeculæ of connective tissue which formed a network, in the meshes of which was a varying number of cells, some probably endothelial, others connective tissue cells, and a great number of blastomycetes. In some parts the cells were quite numerous, in others almost entirely lacking, and here also the connective tissue threads were scarce, leaving a picture of quantities of blastomycetes embedded in a homogeneous, gelatinous mass. (Plate III., Figs. 4 and 5.) Such a mass always surrounds the blastomycetes; sometimes it is not plentiful and difficult to perceive, again particularly prominent and often wrinkled or puckered, perhaps due to contraction caused by the hardening reagents. In the more cellular parts of the lesion numerous giant cells were present. These cells varied much in size and were often phagocytic, sometimes containing several blastomycetes. (Plate IV., Fig. 1.) The blastomycetes varied much in size and were surrounded by a definite membrane often showing a double contour. Within some of the blastomycetes numerous small granules of fat were often seen; others contained only one larger or smaller granule. In numerous sections made through different portions of the lesions, the above histological appearances were constant.

CULTURES. — From the lesion, pure cultures of a blastomycetes were obtained upon potato, and from this transfers were made to a variety of media. Gelatine plates showed white, pin-head colonies in five to seven days which never increased much in size as time elapsed. The surface colonies were distinctly elevated. The growth on serum and agar is white and quite marked after forty-eight hours at 37° C., but ceases to develop very much thereafter whether kept at 37° C. or at the room temperature. The best development occurs upon potato at 25° C., and the growth continues at

this temperature or at that of the room, only limited in extent by the general laws surrounding the increase of organisms in cultures. On this medium the growth is at first white, soon becoming a dirty gray, and after a few days gradually taking on a chocolate brown color. In old cultures the growth upon the less nutritive portions of the medium becomes white and dry, and resembles lime deposits. The color varies quite widely, sometimes remaining a lighter or darker yellow, again assuming the deep brown color almost at once. Such variations are the rule with many forms of *torulæ*.

A MICROSCOPIC EXAMINATION OF PURE CULTURES shows round organisms sometimes slightly oval which vary very much in size. The young forms are surrounded by a delicate membrane which in older forms become much thicker. The blastomycetes are sometimes surrounded by a gelatinous covering (network).⁵ Fat granules are common within the cells, one or two drops may be present, or numerous small granules. Vacuoles are also observed.

Thanks to Dr. Weis, this organism was studied, as far as time would permit, by approved methods,⁶ with the following results:

FERMENTATION TESTS. — Observations were made for ten weeks at different temperatures, viz., 20° to 24° C., 24° to 37° C., and 38° to 40° C., with the result that there was no fermentation in:

Dextrose yeast water,
Lactose " "
Saccharose " " and
Wort.

SPORE FORMATION. — Observations upon spore formation extended over twelve weeks, with the result that no spores were formed on gypsum blocks at the following temperatures:

19° to 24° C.
24° to 27° C.
29° to 37° C.
30° to 40° C.

Besides these important facts which throw this organism into the class *Torula*, the following were noted:

In wort gelatine there was an extensive and ever-increasing surface growth which sank with the gradual liquefaction of the medium.

Wort is the best medium for growth.

The organism is facultative anaërobic.

No mycelia were observed.

There were many "resting cells."

This torula resembles in many ways the two torulæ of Sanfelice and those of Klein and Plimmer, which have been so carefully compared by Weis.⁵ Whether an identity can be established between this organism and the four just mentioned, further study must determine.

INOCULATIONS. — A few rabbits and guinea-pigs were inoculated, some by the direct method with material obtained from the lesion in the horse, others with pure cultures of the torula.

INOCULATIONS BY THE DIRECT METHOD.

Rabbit 1. — A bit of the myxo-sarcomatous portion of the lesion was ground up with sterile water and the suspension thus obtained was injected into the rabbit, one-half cubic centimeter being placed beneath the skin of the abdomen and one cubic centimeter introduced into the ear vein. The animal died fourteen days later. Autopsy. There was no subcutaneous lesion. Both lungs were affected with pneumonia with some abscess formation. Smears from the thick, white contents of the bronchi showed no blastomycetes. The other organs were normal. A microscopic examination of the lungs showed a more or less chronic pneumonia and a few scattered blastomycetes. It is probable that these organisms prepared the way for a secondary infection.

Guinea-Pig 1. The same suspension used as for rabbit 1; one cubic centimeter was injected into the peritoneal cavity, and one-quarter cubic centimeter into the subcuta-

neous tissue of the abdomen. A few days after inoculation a very marked subcutaneous swelling extended over most of the belly. This gradually decreased in size, leaving only a small local enlargement where the needle entered. This animal was killed twenty-eight days after inoculation. Autopsy: In the subcutaneous tissue at the point of inoculation was a firm, irregular, double pea-sized mass, consisting of several translucent, gray nodules. There was a small pea-sized enlargement of an inguinal lymph node near the seat of inoculation, and an inguinal lymph node on the opposite side of the body was slightly enlarged. On the parietal peritoneum opposite the point of injection was a group of about twenty isolated, pin-head, firm, grayish foci, having upon close examination a very delicate honey-combed structure. (Plate IV., Fig. 4.) All other organs were macroscopically normal.

INOCULATIONS WITH PURE CULTURES.

The material used for the following inoculations was prepared by adding a small amount of the original potato culture fourteen days old to sterile water and thoroughly mixing so that the water became slightly cloudy.

Rabbit 2.—One cubic centimeter of the above fluid was injected into the peritoneal cavity and one-half cubic centimeter into the right testis. The animal died four days later of peritonitis. No blastomycetes could be found in the cloudy fluid contained in the abdominal cavity, but a potato culture made from this developed three colonies of blastomycetes. The testis was greatly swollen and hemorrhagic.

Guinea-Pig 2.—One cubic centimeter was injected into the peritoneal cavity and one-half cubic centimeter into the tissue of the right mamma. Two days later there was a swelling the size of a horse-chestnut in the right inguinal region. This gradually decreased in size as time passed and in several places small openings appeared in the skin over the nodule from which there was, especially upon pressure, a yellowish gelatinous discharge containing leucocytes and numerous blastomycetes. The animal was killed fifteen days after inoculation. Autopsy: Occupying the position of the

right mamma was a nodule the size of a nut. It was loosely attached to the abdominal muscles by a caseous mass. (Smears from this showed no blastomycetes, but a potato culture from the same developed several colonies.) The rest of the nodule was myxomatous in character and enclosed an enlarged lymph node. The nodule was surrounded by a decidedly thick connective tissue capsule. The internal organs were normal.

The following animals were inoculated with the original potato culture one month old mixed with water as in the previous experiments:

Rabbit 4. — Twenty minims were injected into the abdominal cavity and five minims into the tissue of the right testicle. The animal was killed ten days after inoculation. Autopsy: A slight amount of clear, light red serum was present in the peritoneal cavity. Cultures from this remained sterile. The right testicle had doubled in size and was immovable in the scrotum. On section there was much edema of the scrotum and the gland was very dark red in color. Cultures were made from this organ, resulting in numerous colonies of blastomycetes. The internal organs were normal.

Rabbit 3. — This animal received twenty-five minims in the subcutaneous tissue of the abdomen and five minims in the right testis. It died eleven days after inoculation. Autopsy: The inguinal lymph nodes near the seat of inoculation were very much enlarged and decidedly hemorrhagic. The right testicle was much increased in size, but not as large as the testicle of rabbit 4. There was also less edema of the scrotum and very slight injection of the gland. Peritonitis was very marked, the peritoneal cavity being filled with a cloudy, flocculent, and slightly reddish fluid; in places there were layers of fibrin. Potato cultures from this fluid remained sterile.

Guinea-Pig 4. — This animal received twenty-five minims in the abdominal cavity and five minims in the right mamma. It died thirty-three days later. Autopsy: In the inguinal region were two distinct nodules, not attached to each other and not adherent to the abdominal muscles. The more

anterior nodule was three and one-half centimeters long and two centimeters in diameter, and consisted of a reddish yellow, translucent, jelly-like mass without macroscopic evidence of any connective tissue capsule. The other nodule was five centimeters long and from one to two centimeters in diameter, and was less jelly-like in appearance. Smears were positive and cultures gave a profuse growth of blastomycetes. The inguinal lymph nodes on the opposite side were enlarged and showed macroscopic evidence of blastomycetic invasion. On the peritoneum towards the median line were small isolated, translucent foci which became more numerous superiorly, soon coalescing and forming a thick, yellowish to light red, smooth, jelly-like layer upon the peritoneum, its greatest thickness being one and one-quarter centimeters. On the omentum was a yellowish-red mass of jelly seven and one-half centimeters long, and from one to four centimeters in thickness. This was more or less rough and nodulated, and in two places so deeply furrowed as to give the lesion the appearance of consisting of three distinct nodules. From this main lesion and following the line of vessels there ran out upon the omentum several strands of reddish-yellow jelly about nine centimeters long and one-half to one centimeter wide. In another portion of the omentum were isolated, translucent, jelly-like foci varying in size from a pin's head to a small pea. Cultures from the omental lesion gave an extensive growth of blastomycetes. Here and there in the mesentery were a few isolated pin-head foci, typical of blastomycetic infection. The mesenteric glands were not involved. The other organs showed no macroscopic lesions, and microscopically the kidneys were free from blastomycetes.

Guinea-Pig 3.—Inoculated with twenty-five minims in the abdominal cavity and with five minims in the right mamma. The usual subcutaneous swelling occurred in a few days, growing very large, rupturing the skin in several places, and discharging quantities of purulent, translucent, gelatinous material filled with blastomycetes. At the time of death the nodule was somewhat smaller than it had been

some weeks previously. The animal died forty-eight days after inoculation. Autopsy: The subcutaneous lesions, including changes in the lymph nodes, were quite similar to those found in guinea-pig 4, as was also the condition of the omentum, though in this case it was less marked. On the peritoneum there was but one split-pea sized focus. On one fold of mesentery were about twenty pin-head foci and one nodule twice the size of a pea. The mesenteric glands were free from invasion. In this animal there were metastases in the other organs. In the spleen were five pin-head to split-pea foci. The right lobe of the liver was firmly attached to the right suprarenal and to the right kidney. The right kidney contained two small foci, the larger split-pea in size, and in the hilus was an elongated gelatinous mass. There were also numerous foci in the lungs.

HISTOLOGY — The microscopic appearance of the lesions produced in the experiment animals by inoculation of this torula is briefly as follows:*

Subcutaneous Lesions. — The nodules formed at the seat of inoculation consisted of delicate trabeculæ of connective tissue closely woven together and forming a fine network. The spaces thus formed were filled with a homogeneous gelatinous mass in which were numerous blastomycetes of different sizes, and rarely a few cells, some probably endothelial, with vesicular nuclei, and others connective tissue cells. An occasional giant cell was seen generally towards the limiting edge of the nodule, but these and other cells were never as prominent throughout the nodule as in the original lesion in the horse. The connective tissue surrounding the nodule varied greatly in amount. Sometimes it was decidedly in evidence, as in the case of guinea-pig 2 (Plate IV., Fig. 3, two weeks after inoculation), or it was exceedingly slight in amount. The delicate fibers of connective tissue which ramify throughout the myxomatous portion of the lesion were often accompanied by small blood-vessels. Twice a *lymph node* was found within the gelatinous nodule.

*Tissues were hardened in 10 per cent formalin or in Zenker's fluid; cut in paraffin and stained by Nichols' method or with hematoxylin and eosin.

It was enlarged and more or less invaded with blastomycetes, which showed their characteristic envelopment interlaced with connective tissue trabeculæ. In these nodes more nuclei were found among the blastomycetes than in the subcutaneous lesions. An occasional giant cell was also present. The advancing edge showed the usual tissue reaction to a foreign body. Lymph nodes outside of the nodule on the opposite side of the body, for example, showed similar changes, all identical in character to the original lesion.

Sections of the nodule resulting from *mammary gland* inoculations were similar to those just described. Gland tissue was difficult to discover, but when found the epithelium was normal.

Sections of the *testicle* (two weeks after inoculation) showed great extravasation of serum, leucocytes, and red blood corpuscles. The glandular epithelium was almost everywhere necrotic, the lumen of the tubules filled with necrotic detritus and spermatozoa. Blastomycetes were very scarce, a single individual being found here and there throughout the organ. Only in two small areas were several, six to ten blastomycetes collected together, and in these regions the usual delicate trabeculæ of connective tissue and the gelatinous deposit about the organisms was evident. Immediately surrounding such foci were epithelioid cells and the usual accompaniments of tissue reaction.

Peritoneum. — The lesions here, whether isolated foci (Plate IV., Fig. 4) or covering the membrane in a thick, uniform gelatinous layer, were identical with those heretofore described, viz., blastomycetes surrounded by a gelatinous material and bound together by a delicate network of connective tissue, such nuclei as were present being those of the connective tissue.

The lesions in the spleen, kidney, and lungs of guinea-pig 3 have not yet been studied histologically, but since they are macroscopically identical with the lesions described elsewhere and unmistakably typical of blastomycetic infection, it is fair to assume that microscopically they will show the same type.

Conclusions.

1. The lung lesion in the horse was caused by a torula; whether identical with Klein's, Plimmer's, or one of Sanfelice's, is not yet determined.
2. Inoculations of rabbits and guinea-pigs with material from the original lesion and with pure cultures of the torula obtained from it, reproduced similar lesions in these animals, and if time permitted, metastases.
3. This torula produces a purely inflammatory reaction in tissues. It does not cause a proliferation of epithelial cells; it may cause a necrosis of them or it may leave them uninjured.
4. The results of my experiments are identical with those of Nichols, though I seem to have been working with a more virulent organism.

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DESCRIPTION OF PLATES.

PLATE III.

FIG. 1. — Photograph of a painting of the lung lesion in the horse. The central, myxomatous, portion is removed.

FIG. 2. — A more cellular part of the myxomatous portion of the lesion in the horse. Note several giant cells, numerous nuclei, and many blastomycetes. Leitz obj. 3, proj. oc. 2. Hardened in 10 per cent formalin; stained by Nichols' method.

FIG. 3. — Another part of same lesion, showing connective tissue trabe-

culæ, degenerated remains of cells, blastomycetes surrounded by a thick mass of jelly more or less puckered. Zeiss D, proj. oc. 2. Hardened in Zenker; Nichols' stain.

FIG. 4. — A very gelatinous portion of same lesion. Leitz 3, proj. oc. 2. Hardened in formalin; stained with hematoxylin and eosin.

FIG. 5. — Same as Fig. 4. Zeiss D, proj. oc. 2. Hardened in Zenker; Nichols' stain.

PLATE IV.

FIG. 1. — Lung lesion of horse. Note nuclei, blastomycetes, and giant cells. (a.) A phagocytic giant cell containing two blastomycetes. Zeiss D, proj. oc. 4. Hardened in formalin; Nichols' stain.

FIG. 2. — Drawing of torulæ from a pure potato culture. Zeiss D, oc. 3.

FIG. 3. — Subcutaneous lesion in guinea-pig fifteen days after inoculation with pure culture. Note the extensive growth of connective tissue to the right. The torulæ are imbedded in a thick gelatinous material which is lacking in the clear areas. Leitz 3, proj. oc. 2. Hardened in Zenker; stained with hematoxylin and eosin.

FIG. 4. — Small focus on peritoneum of guinea-pig No. 1, twenty-eight days after inoculation with material from the lesion in the horse. Leitz 3, proj. oc. 1. Hardened in formalin; stained with hematoxylin and eosin.

FIG. 5. — Subcutaneous lesion in guinea-pig 1. Leitz 3, proj. oc. 2. Hardened in formalin; stained with hematoxylin and eosin.

FURTHER INVESTIGATIONS IN TRANSPLANTATION OF TUMORS.¹

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In a former paper² I published the results of a series of consecutive transplantations into numerous other rats of a sarcoma found in a white rat. I do not need to give at this point a summary of those results, because in the following it will be necessary to compare the previously reported results with the ones obtained in the later work, and in this connection the main conclusions of the previous work will have to be restated.

From March, 1901, until the end of August, 1901, the transplantations described in the former paper were continued. This tumor was, on the whole, carried through about forty generations in the course of twenty months. Almost all of the pieces of the later generations were secondarily infected at the time of the transplantation; nevertheless it was possible to obtain tumors from these transplanted pieces. The number of those pieces which caused (by their secondary infection) putrefaction without tumor growth increased, until the last tumor was infected to such a degree that no tumors resulted from transplanted pieces. The character of these last transplanted pieces remained the same as before — a typical cystic sarcoma developed in each case.

During the summer of 1901 a rat was obtained from a source not identical with the one from which the sarcomatous rat just mentioned had come, which was believed to be pregnant. Her abdomen was greatly enlarged. This rat died unexpectedly during the night, and on opening the abdomen the next day a large tumor was found which distended the abdominal cavity. It was fixed to the mesentery. Six pieces were transplanted into different rats, but without

¹ Read March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists at Cleveland, Ohio.

² On the Transplantation of Tumors. *Journal of Medical Research*, Vol. vi, No. 1.

results in any case, although the subjects were under observation for a considerable period. On microscopical examination the tumor was found to be an adeno-carcinoma, probably originating from the pancreas. No metastases were present.

Early in November, 1901, I obtained, through the kindness of Dr. Barrett, in Danvers, Mass., a full-grown rat with a tumor pendent from the neck, which was about seven centimeters long, and measured four to five centimeters in the other dimensions. This tumor hung down so far from the neck of the rat that it touched the ground. It felt solid and moderately hard. About two centimeters from the neck on the pendent portion of the tumor there was a constriction encircling it and dividing it into two lobes. During the operation the tumor was found to contain a small central cavity filled with straw-colored fluid. The color of the tumor in this region was white. Besides the small central cavity, no cysts were macroscopically present. In trying to enucleate the tumor at the neck, it was found that it had surrounded important blood vessels and nerves of the neck, and this fact prevented the possibility of saving the life of the rat. At this time it was observed that the tumor had a somewhat different color at the upper part near the neck, above the constriction, but no especial significance was attributed to this fact, and no pieces from this upper part were transplanted. Later on, microscopical examination showed that the part near the neck (probably the part above the constriction) was adeno-carcinomatous, but that the main mass of the tumor which was white in appearance was a sarcoma. In one lung a small nodule was found, which showed sarcoma-like tissue with mitoses in each section around a blood vessel. It is very probable that we have here a beginning metastasis of the sarcomatous part of the mixed tumor of the thyroid. Two rats were injected intraperitoneally with three cubic centimeters each of the serous fluid found in the central cavity, and a guinea-pig was treated in the same manner with two cubic centimeters of the fluid, with negative results in each case. Six pieces of the white or sarcomatous portion

of the tumor were transplanted into several rats, subcutaneously, intraperitoneally, and into the tunica vaginalis of the testis.¹ After two to three weeks most of the transplanted pieces began to grow and developed large tumors. This tumor has since then been transplanted through eight generations, and is still growing. Some of the observations made in connection with these transplantations, and the result of some experiments, will be stated later.

Further transplantations were made from a tumor found in a rat received from a source different from that from which the other rats were obtained.

In the latter part of December, 1901, I received a rat with a tumor in the region of one of the mammary glands, about three centimeters in the longest dimension, and one and one-half to two centimeters in the others. The tumor seemed to be solid and was hard. For eleven days the rat was kept under observation. During this time the tumor became somewhat softer and smaller. The microscopic examination showed that it was an adenoma of the mammary gland. January eighth, 1902, one piece of the tumor was transplanted into the other side of the abdominal wall of the same rat (subcutaneously), four pieces subcutaneously and intraperitoneally into other rats, and a piece of the original tumor was left in situ. February fourth a section was taken from the piece transplanted into the same subject, and also one from a transplantation into another animal (a male rat).

The macroscopic difference was not striking. The microscopic examination, however, which shall be reported later, showed a striking difference. The two pieces remaining in the rat, in which the tumor was originally found, began to grow rapidly, until by February sixteenth both pieces had acquired perhaps eight times their original size. February sixteenth this rat gave birth to a young rat. February twentieth her two tumors (the original adenoma and the transplanted piece) had simultaneously become somewhat smaller. February twenty-

¹ It is intended to discuss this tumor somewhat more fully in another connection. Here only so much is given as is necessary for the present purpose, to indicate the source of the transplanted pieces.

second the rest of the piece transplanted into one of the other animals, from which a piece had been excised February fourth, was removed; also pieces from the two tumors of the originally affected rat, and on February twenty-sixth the piece transplanted into another rat intraperitoneally was taken out for examination. None of these pieces, except the two in the original tumor rat, showed any growth on examination by the naked eye. Simultaneously with these transplantations, others were performed with a tumor found in a rat in the region of the thyroid, which closely resembled the tumor used by myself in my former work, and which was, like this former tumor, found in the Laboratory of the Chicago Policlinic. Dr. Maximilian Herzog kindly gave me a few pieces of this tumor for transplantation. Just as in the previously reported investigations,¹ the tumor, after excision, recurred in several distinct nodules. In this case, however, a metastasis at a different part of the body was also formed. This metastasis was found in the groin on the left side, perhaps originating in a lymph gland. It consisted, as did the original tumor, of cysts filled with a gelatinous material formed through degeneration of the sarcomatous mass. The nodules at the neck also contained much degenerated material. Therefore tumors transplanted from these did not grow, but other pieces transplanted from the original tumor did grow. From one tumor which had ceased to grow a piece was transplanted, November twenty-sixth, into another rat, and this had developed a tumor December fifteenth. This confirms similar experiments reported in my former paper.

After having given a general survey of the experiments carried out, it will be necessary to discuss some of the results obtained.

It may first be asked, Are the new growths used for these experiments sarcomata or are they granulomata? Their structure is that of a spindle-cell sarcoma, with a tendency to form cuboidal or polygonal cells, which polymorphism, as is well known, is very frequently found in sarcoma. Two of these sarcomata showed a very characteristic cyst formation

¹ L. Loeb. On the transplantation of tumors. *Jour. of Medical Research*, Vol. vi, No. 1, 1901.

with gelatinous contents, which has only been observed in true tumors. All these original growths did grow indefinitely. They showed a large number of mitoses. These characteristic features were, on the whole, also found in the transplanted piece. These usually had an infiltrating growth, and, just as the original tumor, they invaded and destroyed the neighboring tissues and organs. In the two cases in which the animals lived some time after extirpation of the primary tumors, multiple recurrences took place, a fact frequently observed in tumors. Contact metastases were frequent, just as they have been described in tumors, but also metastases formed at places far off from the original tumor, where only the lymph or blood stream could convey the tumor-producing agencies. In the mixed tumor of the thyroid a small nodule was found in the lung. In the second cystic sarcoma a metastasis formed in the left groin. We must take into consideration that in man also some tumors vary considerably in regard to their formation of metastases, and, further, that time plays a part in this process. These tumors are usually not of very long standing — several months, perhaps. Rats with successfully transplanted tumors usually live not longer than two to three months after the operation. Metastases (also in man) usually develop only after the original tumor had existed for a longer period. A very important proof that these new growths are real tumors is found in the fact that one of them was found in combination with an adeno-carcinoma, forming the larger part of this mixed tumor.

On the other hand, these tumors could never be successfully transplanted into any other species of animals except the one in which it was originally found. Guinea-pigs, animals so susceptible to tuberculosis of different origin, remained absolutely unaffected by these tumors. Certainly none of the ordinary microorganisms can be the cause of these new growths, and if a microorganism is the cause of it, this microorganism would not be very different from the microorganism or the microorganisms which cause the sarcoma in men. On the other side, no element was present in these tumors

which spoke against their sarcomatous nature. Small round cells resembling (or identical with) lymphocytes were quite frequently present, especially at the progressing margins of the tumor; an occurrence quite common in tumors, but the tumor did not consist of these small round cells. Giant cells were only found in these tumors if foreign bodies were introduced into them experimentally or accidentally. *There can be no doubt, therefore, that these new growths must be classed among the true sarcomata*, whose every characteristic they have. It is probable that transplantation of sarcoma from man to man would give similar results.

The mammary tumor will be discussed at the end.

One of the results of the first series of consecutive transplantations already reported was that the morphological character of the new formations remained, on the whole, unchanged. We always had to deal with a spindle-cell sarcoma whose cells had frequently a tendency to assume a cuboidal shape, which, by myxoid degeneration, formed cysts with a typical arrangement of the cells adjoining the cysts. The tumor cells frequently had a radiating or concentric position around the blood vessels. Twice all cells assumed a large cuboidal shape, so that the character of endotheliomatous tumors resulted. Through degeneration of the cells as a result of thrombosis of the blood vessels pyknosis of the nuclei and shrinking of the cell bodies took place, with subsequent liquefaction. The cells around the normal blood vessels were preserved. *The width of this zone of cells around these blood vessels was almost identical in all cases, hence this could serve as a means of determining how large the nutritive area of a blood vessel on cells is.* In this way a papillary character of the tumor resulted secondarily.

It will now be of interest to check these results by examining the character of tumors which were transplanted from a somewhat different source. This was accomplished through the second series of consecutive transplantations of the sarcomatous part of the mixed tumor of the thyroid. This very large tumor contained a small cavity in the center, produced

through degenerations of the cells, but the great mass of the tumor was free from large cysts. The characteristic of these tumor cells was that they did not get so large as the sarcomatous cells of the first or of the second cystic tumor. The peripheral protoplasm of the cells formed fibers. In the more central part of the cell, a somewhat fibrillar character was also produced through vacuolization taking place. This vacuolization, produced by some hydropic or myxoid change, occasionally went so far that small areas of the tissue became rarefied, and that small cavities were occasionally formed. No large cysts were formed in this way, however. The myxoid *simultaneous* dissolution of a whole area of cells, which had previously not produced such well developed fibers, did not usually take place. In this tumor, also, cysts were formed by the second process, just the same as in the first tumor. After primary thrombosis of blood vessels, probably often produced by infection with bacteria, the cells surrounding the blood vessels shrank, the nuclei became pyknotic, and in such tissue liquefaction frequently took place. (This liquefaction is not dependent on leucocytes, and must be produced by proteolytic ferments, probably present in the connective tissue cells (sarcomatous cells), perhaps present in the liquid of this necrotic area.) In this way, also here, cysts were occasionally formed, just as in the first tumor, and possibly at times also by hemorrhages. This absence of large myxoid cysts (cysts of the first kind) in the second series of tumor transplantations was observed throughout the whole series of eight generations. All the tumors of this second series had a much more solid character. In accordance with the fact that the fibers of this second series of tumors were more solid, is the other fact that in some areas of the primary tumor the cells and nuclei were lost, and the fibers only remained without much myxoid change. The same observation could afterwards be made in some tumors derived from this primary tumor, following the transplantation of several generations.

Occasionally we found in the second series of transplantations cells arranged concentrically about the blood vessels,

but they were not arranged in a radiating way. Blood vessels with areas of preserved tumor cells around them were also formed in this case. Such pronounced new formations of cells, with an endothelial character, were not observed in this series. The character of the second cystic tumor used for transportation was, on the whole, the same as the first one, as was also the metastasis formed in the groin, and the character of the pieces transplanted from it. These same cysts formed always through myxoid degeneration. In the first cystic tumor we found frequently hydropic tumor cells in the cysts, not rarely in the process of mitosis. These cells were not absolutely missing in the series transplanted from the mixed tumor, but they were very rare. They were also not as frequent in the series derived from the second cystic sarcoma as in that obtained from the first cystic tumor.

It is known that in metastases, on the whole, the character of the original tumor is preserved, although slight deviations occur, but metastases usually only represent the first, or perhaps the second, generation of tumor cells, and in metastases the individual remains the same, introducing a constitutional element which might here play a part (as under certain circumstances, to be shown later on, is really the case). But at these transplantations in which about forty and eight generations respectively were investigated, animals of different age, sex, and even hybrids were used. *Still the character of the tumor remained unchanged, for not only was a sarcoma always reproduced, but even the details of the growth and degenerative changes of the cells were preserved with certain limits of variability which were also preserved, although all the original tumors were derived from the connective tissue near the thyroid (or more probably the parathyroid).*

It must, however, be stated that these differences between the two original tumors are more of a quantitative than of a qualitative kind, because myxoid changes took place also in the second tumor, although to a less degree, and because here also the cells have more or less of a tendency to assume

a cuboidal shape; moreover, the first tumor frequently had the intercellular substance well developed. These *quantitative* differences were preserved throughout both series of transplantations.

The question arises here, Have we in these experiments to deal with transplantations of cells which carried with them that so far unknown agency which makes these cells reproduce themselves almost indefinitely, or have we simply to deal with the injection or inoculation of microorganisms which are the cause of sarcoma. In my first paper I declared it to be very probable that we have to deal with a real transmission of sarcoma cells. Since then I have continued, in the second series of transplantations, to investigate the fate of the transplanted piece immediately following the operation. The nuclei of the cells situated in the central part of the piece in this series, just as in the first, became pyknotic and the cells gradually necrotic, but the peripheral transplanted cells remained alive for the first few days and then mixed with the growing connective cells of the surrounding area. Additional experiments were made to determine more exactly the origin of the cells in the peripheral part of the tumor. Pieces of agar were put into rats and examined at different periods. After twenty hours mostly leucocytes are around and in the agar. At this time, however, the cells in the peripheral part of the tumor are already present. These cells also show perfectly the character of the sarcoma cells. Also in the agar we find at some places after forty hours many round cells which cannot be distinguished from connective tissue cells; but at certain points only are they in large numbers. There can, therefore, be no doubt that these peripheral cells (which are only present where real tumor tissue is transplanted, and not where adjoining tissue was transplanted) are really the transplanted tumor cells which soon develop under favorable conditions for their further growth. In this series transplantations and injections of tumor fluid were also undertaken into guinea-pigs, white mice, and rabbits, as well as into rats. Only in rats were positive results obtained. Now, it is a well-known fact that transplantation of

cells into animals of a different species are usually unsuccessful, and this might easily account for the impossibility of producing a tumor in another species. The above-mentioned fact, namely, that the character of the sarcomatous connective tissue and its difference in the two series were preserved so well throughout, has also to be taken into account. *All these facts make it very probable that besides the tumor-producing agency the tumor cells themselves were transplanted.* The last-mentioned fact, however, seems to be remarkable still from another point of view. We might assume that in the two series we had to deal with microorganisms differing somewhat from the beginning, and preserving this difference through many generations, or, on the other hand, with connective tissue of somewhat different derivation and character in each original tumor. The variability of connective tissue in certain limits, however, has been demonstrated. We see that both sarcomata from which these two series of transplantations were started had some distinctive characteristics which were, in all details, preserved during the large number of transplantations. We see further that these differences are rather of a quantitative than of a qualitative character, slight myxoid changes, for instance, also taking place in the second series, the cells of the second series also having a tendency to assume cuboidal shape, and occasionally being arranged concentrically around blood vessels. It would, therefore, be very difficult to assume that both sarcomata are derived from absolutely different kinds of cells; for instance, the one from an endothelial cell of a blood or lymph vessel and the second from an ordinary connective tissue cell. Both tumor cells are somewhat different, but these differences are not absolute, they vary, and according to our present knowledge, the sarcoma cells of these two series do not correspond to two fixed types of cells. (Perhaps later on it will be found that there really exist different types of connective tissue cells corresponding to the two types described here, and possessing the same breadth of variability.) There is still another fact which makes it impossible that in these two series we had to deal with two different types of

cells. In the first series we saw that the tumor in its beginning was formed by ordinary connective tissue cells. Only secondarily definite arrangements around blood vessels became marked, and the two tumors resembling endotheliomata were among the older tumors examined microscopically. If, however, endothelial cells were transplanted in the first series, a tumor-like new formation of blood or lymph vessels ought to have been expected to take place first, especially as in the first series of transplantations injection of single tumor cells was sufficient to produce sarcomata. It would be easier to explain these facts if we would assume that the different shape of these cells, and especially their relations to the blood vessels, are not determined by genetic, but rather by nutritive and functional relations.

The facts with regard to the structure of these transplanted tumors deserve some consideration, if we try to explain the differences of certain connective tissue tumors. There is not yet any classification universally adopted, but the majority of writers perhaps distinguish besides the ordinary sarcoma, perithelioma, lymph endothelioma, and hemendothelioma. Now, in the first series of transplantations, the tumors frequently showed the character of a perithelioma. We would have to assume, then, with some pathologists, that these cells were originally all derived from the adventitia of blood vessels. In other cases the tumor had a perfect endotheliomatous structure. In these cases it would have to be assumed that the tumor was derived from endothelial cells of blood or lymph vessels. Usually, however, we had to deal with an ordinary spindle-cell sarcoma, in which the cells not infrequently had a tendency to become cuboidal. In four cases it was possible to produce these tumors through injection of their cystic fluid, in which tumor cells could be found often in the process of mitotic division. In these cases, therefore, the tumor probably originated from a single cell. In the beginning these cells usually produce a multiplication of ordinary connective tissue cells. If these cells, which afterwards produce peritheliomatous or even endotheliomatous structures, were adventitial or endothelial cells, one ought to expect them to

produce, from the start, blood or lymph vessels or structures similar to the adventitia of lymph or blood vessels, but this is not the case. For some tumors, therefore (especially for some peritheliomata), we may consider the possibility of another way of explaining these structural peculiarities. We have seen already that the nutritive influence exerted by blood vessels on a certain surrounding area of connective tissue cells produces certain structural appearances of a close relation between the blood vessels and connective tissue cells. This relation is not a genetic one, but a functional one. In a similar way many of these appearances of a parallel or radiating arrangement of sarcomatous connective tissue cells to blood vessels, the more or less cuboidal form of the cells, with more or less production of intercellular substances, may be functional conditions, dependent on a series of factors of nutrition, and of interdependence of different varieties of cells, and may not be dependent in every case on a genetic relation; such an explanation would presuppose that only cells derived from the adventitia of blood vessels assume the radiating arrangement around the capillaries. These remarks are only intended as suggestions so far, but certainly these experimental facts must be taken into account in determining the cause of the different structure of connective tissue tumors. Some variability in the relation of sarcoma cells to blood vessels in the original tumor and in metastases respectively has also been described lately by Low and Lund.¹

Although, as stated before, the greatest part of the transplanted piece becomes necrotic, I sometimes looked in vain for the transplanted piece in tumors which had started to grow. In my former paper I could not make any definite statement with regard to the question whether the growing sarcomatous connective tissue penetrates into the necrotic part of the transplanted piece or not. In the new series of transplantations I have been able to observe that such a penetration really does take place, and that in this way a part, at

¹ Low and Lund. Tubular perivascular sarcoma. *Journal of Medical Research*, Vol. vii, 1902.

least, of the necrotic piece becomes resorbed. This was very clear in a piece fourteen days after transplantation. Simultaneously with the advancement of the growing sarcoma, liquefaction of the necrotic piece takes place, and in the beginning, in the second series of transplantations, just as in the first one, cysts may be formed where the necrotic tissue becomes resorbed. Also, in this case, it is not unlikely that the sarcomatous connective tissue cells exert a dissolving influence by proteolytic ferments.

In my first paper I stated that pieces infected with putrefactive bacteria during the process of transplantation developed tumors in several cases. The same observation was made in this latter series. The rapidly growing cells seem to be very slightly sensitive to the substances produced by these bacteria. If, however, during their rapid growth a number of cells get into unfavorable conditions with regard to their nutrition, and as a result of that begin to degenerate, the bacteria find a good soil for growth, and their influence increases gradually. It is a well-known fact that healthy granulation tissue shows a not inconsiderable resistance towards the action of bacteria. We, therefore, find the development of the bacteria usually in the inside of the tumor, where the degeneration of the tissue is most rapid, the nutritive conditions being most unfavorable.

In the former series of experiments I succeeded in four cases in producing tumors by the injection of the cystic fluid. I injected fluid from the second cystic tumor with negative results. The fluid found in the original mixed tumor of the thyroid (or parathyroid?) was injected into different animals without positive results. Later on, in a number of cases, the more solid parts of these sarcomata transplanted from the original mixed tumor were minced, and under addition of normal salt solution converted into a pulp in a sterilized mortar. The fluid thus obtained was injected into rats, mice, and guinea-pigs. Although repeated in a number of cases, the results were negative. In one case the possibility of a positive result cannot be excluded, a tumor growing subcutaneously four weeks after injection of fluid, but it is more

probable that another piece had previously been transplanted subcutaneously in this rat, owing to the fact that the number of rats at my disposal at that time was somewhat reduced, and one rat may have been used for a second experiment. In all former cases after intraperitoneal injection of fluid the growth was intraperitoneal, and not subcutaneous, as in this case. But certainly in all other cases no tumor resulted after injection. At the same time I made injections of fluid filtered through filter paper, asbestos, and in one case also through a Berkefeld filter. These latter filtrations were done in the Gratwick Laboratory in Buffalo. In no case, of course, was a positive result obtained, the control experiments of unfiltered fluid having proven unsuccessful. *Without positive control experiments negative results of injection of filtered fluid do not permit of any conclusion in regard to the character of the tumor-producing agency.* Should control experiments be successful, filtration through different kinds of filters might provide us with means to get a more certain insight into the character (and perhaps size) of the tumor-producing agency.

In the first series of transplantations after introduction of a piece into the abdominal cavity metastases were formed occasionally in the wound in the abdominal wall, becoming apparent after about three to six weeks. One similar occurrence took place in the second series, transplanted from the mixed tumor. Many metastases in the abdominal cavity of a piece grown through the peritoneum were observed in one case, just as such multiple metastases were formed in the previous series. While in the first series a formation of independent secondary nodules of the pieces introduced into a subcutaneous pocket was not rare, such a formation never took place in the second series. There is probably the same reason for this as existed for the want of success of the injection of tumor fluid into the peritoneal cavity in the second series. In the first series we found frequently hydropic cells, often in mitotic division, in the cysts. They were very rarely or never found in the tumors of the second series, and it is probable that the presence or absence respectively of these

cells in the two series determined the result of the injection of the fluid, or of the formation of secondary nodules after introduction of the pieces.

Just as in the first series, so also in the second, secondary nodules were formed, if ulceration had taken place, or even only infection in some part of the tumor.

With regard to the rate of growth, the description given for the first series holds good, on the whole. From the ninth to the fourteenth day after the transplantation the growth of the piece usually became apparent; but usually no large cysts were formed in these series, and therefore the growth of the tumor proceeded more evenly, and if no infection and ulceration took place the tumor reached half the size of the rat, or even more. After six to ten weeks the animals usually died in a very anemic and weak condition.

The question, are there individual differences in different rats with regard to the facility with which the transplanted pieces grow, or are there rats which are naturally immune against tumors, is a very interesting one, but very difficult to decide, because a number of factors complicate this problem. I said in regard to this problem in my former paper: "Old and young, female and male rats were used in these experiments. It was possible to get positive results from all kinds. It may nevertheless be the case that a certain difference exists between different varieties of rats, that it might be more difficult to make tumors grow in certain rats than in others. This will be determined in further experiments." Even in a hybrid between a white and a wild gray rat tumors were successfully transplanted. I noticed that in several rats tumors did not grow after repeated transplantation; further, that in the majority of cases, if two pieces were transplanted into one rat, either both pieces began to grow, or neither of them. These two facts pointed to individual differences. But the complicating factors which make an absolute decision of this question difficult are the following:

1. Different parts of the tumor have a very different vitality. Even apparently healthy parts may microscopically show the beginning of degenerative changes.

2. The size of the transplanted piece plays a part, larger pieces promising better results than smaller ones.

3. Infected pieces, even if the infection has not yet produced suppuration or putrefaction, do not usually give as good results as sterile pieces.

4. Local factors play a part, as my experiments published in my first paper demonstrate. A tumor ceasing to grow begins to grow again, after excising a piece and removing a piece of the surrounding connective tissue capsule. Or a piece taken from a shrinking tumor and transplanted into another part of *the same animal* produces a rapidly growing tumor. In the second series a tumor ceasing to grow developed a tumor after excision of a piece and transplantation into another animal. Where so many complicating factors are present the facts mentioned, namely, that an animal did not develop a tumor after two or three transplantations, or that either both or none of the pieces transplanted into one animal grew, are not sufficient to decide this question definitely. The second fact might be explained in this way: that usually two pieces which adjoined each other and were therefore in a similar condition were transplanted into one animal, and that for that reason the fate of the two pieces in the same animal was identical. It is possible to transplant these tumors into so many animals that it is not likely, although not impossible, that marked individual differences do exist. But this could only be proven by a larger number, perhaps seven to ten transplantations into one apparently refractory animal, and an equal number of control transplantations with similar material into another animal.

Similar difficulties present themselves if one wants to decide whether in the series of succeeding transplantations an increase or decrease of virulence takes place. Several facts can be stated in this connection. In the first place, the time after which the transplanted tumor piece began to grow remained about the same throughout. There was, in the first and second series, after forty and eight generations respectively had been transplanted, no marked difference present in this respect. The pieces could in the later periods of the series

reach the same size as in the beginning. But the number of successful transplantations became smaller in the end, and larger pieces had to be transplanted to produce a successful result. It is, however, not possible to interpret this directly as a decrease of virulence caused by the large number of generations already transplanted, which would cause either a decrease in the power of propagation of the sarcomatous connective tissue cells, or a decrease in the power of the tumor-producing agency. There is also a complicating factor present — secondary infection of the transplanted pieces — which often took place.¹ This, in a number of cases, if the number of putrefactive bacteria present was too great, prevented the growth of the transplanted piece, although the piece from which this section was taken may have grown before. This constant activity of bacteria in many pieces may produce a gradual decrease in the virulence of the tumor.

In my former experiments I noticed that, while the original tumor had a comparatively slow growth, the transplanted pieces frequently grew much more rapidly. The same observation was made in the mixed tumor.

Another observation with regard to the rate of growth of the tumors is the following: If a piece is excised from a tumor, the rest frequently continues to grow, but sometimes, especially if ulceration took place first and a very large piece was removed, very firm tumor nodules are formed afterwards, which frequently do not grow. It appears that the surrounding connective tissue capsule prevents further growth.

We must take into consideration that probably in all of these experiments we very likely have to deal with a complicated process. We probably transplant connective tissue cells plus the tumor-producing factor, which ultimately is a chemical, physical, or physico-chemical agency, whether the intermediary agency be a microorganism or not. Now, there might very well exist an individual predisposition or immunity of certain connective cells of one animal for this tumor-

¹ The operations had to be done under very unfavorable conditions, from a surgical point of view, and thus frequently infection was caused, although all means which could be taken were used to carry out the work aseptically.

producing agency, but, on the other hand, there need in the same animal not be any special immunity or predisposition for the growth of other connective tissue cells, which are already under the influence of this tumor-producing factor, and which have been transplanted into this animal. Again, there might be in an individual animal no immunity against the tumor-producing factor, but an immunity against the growth of transplanted cells, as is actually the case in animals of a different species, or which might perhaps be induced in animals of the same species by immunization with cells or cell products, as v. Dungern claims to have accomplished for ciliated epithelium. On the other hand, on the supposition that certain cytotoxins exist in an animal for the cells of a different species, an anticytotoxin might make the growth of the sarcomatous connective tissue cells of the different species possible, provided there did not exist simultaneously in the different species an immunity against the microorganisms, which *perhaps* is the cause of these growths.

It may serve some purpose to present the different possibilities of interpretation of certain facts, but of more value are experiments which throw some light on the character of the factors at work, or which open a way for experimentation which promises to bring us nearer to this insight. With these intentions the following experiments were undertaken. Although the tumor-producing agency connected with the sarcomatous connective tissue cells has not yet been recognized or isolated, this factor must be present in the tissues, and by subjecting the tissues to different experimentation before transplantation we might get some knowledge of the conditions under which this agency will still be active, and under which it will lose its vitality, just as we know many facts about the ferments, although no ferment has yet been isolated, or as it would have been possible by experimentation with tuberculous sputum to get some insight into the character of the tubercle bacillus.

The following experiments were made:

Experiment I.—One animal, with a large tumor (transplanted) died during the night, January tenth, 1902. The

next day, about twelve hours after the death of the animal, pieces were transplanted into three animals. January twenty-eighth, 1902, two transplanted pieces have developed tumors. One of these tumors is very large. After having finished the experiments published in this paper, I saw a paper by Velich,¹ in which he reports the successful transplantation of a sarcoma found on the leg of a rat, into other rats, through nine generations. Velich also succeeded in an experiment similar to the one just described, transplanting a piece of tumor successfully after having kept it for twenty-four hours, probably at room temperature. Velich, however, takes it for granted that no transplantation of tumor cells, but only of microorganisms, took place, which, of course, is not proven or even not probable. But after the results published here, and in my previous paper, there can be no doubt that Velich's experiments deserve to be regarded as transplantations of true sarcomata.

Experiment II. — Two pieces of a large tumor which had a putrid nucleus in the center (the remaining part of the transplanted piece) were kept on ice for twenty-four hours, and one piece was kept at room temperature from January twentieth to January twenty-first, 1902. These pieces were transplanted (cut into smaller pieces) into four rats. The piece which had been kept at room temperature became gangrenous, and did not cause a tumor. February eighth, 1902, three tumors were growing from pieces which had been kept on ice. The microscopic examination of one of these tumors showed the typical structure of the tumor with mitoses and some myxoid degeneration.

Experiment III. — From a tumor which was beginning to ulcerate in the center (which therefore was infected with bacteria), pieces were taken February third, 1902, and kept for two days on ice, and one piece was kept at 44° Celsius. February fifth the pieces kept on ice were transplanted into three rats, and the one piece kept in the thermostat was also transplanted, but only caused putrefaction. February twen-

¹ Alois Velich. Wiener Med. Blätter, 1898, N. 45 u. 46. Beiträg. zur Frage nach der Übertragbarkeit des Sarcoms.

tieth, from the pieces kept for two days on ice two tumors have developed in one animal, one of which shows the result of infection with putrefactive bacteria at one place. February twenty-sixth one of these tumors has grown very large.

Microscopically this tumor shows the typical structure with very many mitoses, at some places cells and nuclei have disappeared and only connective tissue fibers remained, just as in the original tumor.

Experiment IV.— Pieces of tumor, after having been placed on ice for five days from February third to February eighth, were transplanted into four rats. February twentieth, one piece growing, although ulceration had taken place at one part of the tumor. February twenty-third, two other pieces growing. Microscopic examination of one of these pieces showed typical tumor tissue with mitoses and some myxoid degeneration. In another piece, where the animal was cold (in a dying condition) for perhaps one day, no mitoses could be seen, but the typical structure of the tumor was present, with polygonal cells, and some myxoid changes.

Experiment V.— A piece kept from February eleventh to February fourteenth, three days, at variable temperature, does not grow. We therefore see that pieces kept on ice for five days caused, after transplantation, tumor formation, and we can therefore state that the tumor-producing factor does not materially lose in power, after having been kept for five days at a temperature of about two to four degrees Celsius. Less certain, but probable, is that the sarcomatous connective tissue cells also remained alive for this period. This latter fact agrees with the results of Wentscher,¹ Grohé,² and others.

Although it is very probable that sarcomatous connective tissue cells remained alive for this period, and were transplanted after five days, still it is not absolutely certain. But it is certain that the tumor-producing factor was not killed or even materially attenuated in this time, and this is a result which could not be foreseen. I have not made further

¹ T. Wentscher. *Ziegler's Beiträge*, 1898.

² B. Grohé. *Virchow's Arch.*, Bd. 155.

experiments yet, in which I tried to keep pieces on ice for a longer period, but I kept the pieces in different liquid substances for varying periods before transplanting them. Most tumors, however, were, when I started these experiments, already so severely infected that even the control pieces did not grow. *Negative results, therefore, do not prove anything.* A continuation of experiments on these lines, however, promises to give us still further facts with regard to the sarcoma-producing factor.

Experiments were begun on the influence of the Röntgen rays on the subcutaneous tumors with the hope of learning something about the character of the changes produced by the Röntgen-rays. About twenty hours after one exposure of ten minutes there was a considerable number of mitoses present in the tumor cells. After seven exposures of ten minutes each, in the course of about eleven days, mitoses were also present. The tumors continued to grow, and pieces were successfully transplanted from the treated tumor. Degenerative changes were present in the center of the tumor, but such changes take place in many tumors without exposure to Röntgen-rays. They were, however, perhaps increased by such exposure. The influence of a more persistent treatment will be tried later on.

Cultures on the ordinary culture media have so far given only negative results, provided no secondary infection with bacteria was present. Different stains used did not show any of those bodies or cell inclusions frequently seen in carcinoma.

I will now give the results of the transplantation of a tumor found in the region of the mammary gland of a rat.¹ The tumor had attained its largest size, almost the size of the head of a rat. Microscopically it consisted of gland structure. No lumina, or very few only, were present in the alveoli of the original tumor. Many mast cells were situated in the connective tissue.

The mast cells were mostly well filled with granules, in

¹ Thus far I have not been able, since finding this tumor, to get a pregnant rat for a comparative study of the changes in the normal mammary gland and in the tumor.

certain places some granules being distributed in the neighboring tissue. Their nuclei were small, vesicular, occasionally with one or two chromatin granules. Only one mitosis was found in the connective tissue, none in the gland cells. Such was the condition January eighth, 1902, when pieces were transplanted into the same and other rats. February fourth, 1902, the piece transplanted into the same rat showed, on section, the gland structures well preserved, even an increase in gland tissue. At many places numerous mitoses were present, not only in gland cells, but also in connective tissue cells, at such places as were near the growing glands. In the alveoli a lumen is usually present, sometimes filled with a homogeneous mass, staining with hematoxylin. The gland cells, as well as many connective tissue cells, are larger than in the original piece, January eighth. Mast cells are present, but perhaps not so numerous as in the original piece. Many of them have only a few granules; near by are nuclei in the connective tissue, which have the same size and appearance as the nuclei of the mast cells, but they are not surrounded by any granules. The original tumor at this period showed about the same character as the transplanted piece just described. At this time the rat was pregnant, and the original tumor and the transplanted piece were in the beginning of their growth. In some cases isolated gland cells may have been present in the connective tissue, and some of the mitoses apparently found in connective tissue cells may really have been mitotic divisions in such gland cells. This explanation, however, does not apply to all mitoses found in the connective tissue. From now on, until February sixteenth, both pieces, the original and the one transplanted into the same rat, grew rapidly, and on February sixteenth, when the rat gave birth to a young rat, the two pieces had reached about eight times their original size. From now on, until February twenty-second, the two pieces remained stationary, or perhaps decreased slightly. Pieces removed at the latter date for microscopic examination showed, in the original tumor, the gland structures preponderating considerably over the connective tissue; only an exceptional mitosis was found. At some places gland tissue

was perishing, and at such places also occasionally a loss of the connective cells took place. Mast cells were present, many of them with only a few granules; other cells were situated near them, showing a well-developed cell body similar to the one found in mast cells; identical nuclei, but no granules.

Some gland structures were without lumina. The piece transplanted into the same rat showed at this period identical changes; the destruction of gland tissue and of connective tissue cells, however, was not so apparent.

The fate of two pieces transplanted into two other rats was different. One piece transplanted into a male rat was taken out February fourth, and a second part of the same rat February twenty-second. In the first piece the greatest part of it had become necrotic. The nuclei were pyknotic, and the cells had shrunk. At other places the nuclei were lost. In this necrotic part the mast cells were filled with granules; many of the granules were clumped together. Almost all the mast cells were filled to such a degree with these granules that the surrounding area of connective tissue was also filled with them. At other places the cells had broken up, and masses of granules were free in the connective tissue. Very few mast cells were normal; the majority of them were on the point of being broken up. At the margin some glandular cells were preserved, and even processes of growth were taking place, and mitoses could be seen. At some places also normal mast cells could be seen.

February twenty-second, all glandular structures had disappeared in the center. Homogeneous connective tissue was left, with much yellow pigment, as the rest of the gland cells. Some new-formed blood vessels penetrated into this old connective tissue. No mast cells were present, with exception of the periphery, where the surrounding connective tissue was growing. Here some normal mast cells are present, and also some cells which are perhaps preserved gland cells. The mast cells in the center of the piece have therefore disappeared by disintegration in the period from February second to February twenty-fourth. A similar picture presented itself in a piece transplanted into the abdominal cavity of a rat, and taken

out for microscopical examination February twenty-sixth. Only connective tissue without cells was left; yellow pigment was present. This pigment was probably derived from the gland cells. At no time was milk obtained by cutting into this tumor, neither during the time of pregnancy nor of lactation of the mother rat, in which the tumor was originally found. If we take into consideration this lack of function, the relatively enormous size of the tumor, and some peculiarities of its structure, we may call it an adenoma, although it resembled very much the structure of the normal gland. We see that in this case a great difference existed in the fate of the transplanted pieces, dependent upon the animal into which the pieces were transplanted. Transplanted into the same animal, which at the time of transplantation was either in the beginning of pregnancy or soon afterwards became pregnant, the whole gland was preserved, and grew after some time. How much each of these two factors, namely, (first) transplantation into the *same* animal, and (secondly) the pregnancy of this animal, had to do with this result, must be investigated in further experiments.¹ In other animals the central part of the transplanted piece became necrotic, but in the peripheral parts some gland cells were preserved, and even showed processes of regeneration with mitoses, which, however, in this case did not lead to any permanent growth. In a similar way only the peripheral parts of the transplanted sarcomas grew. The remarkable fact is that in the first case the whole gland was preserved. Some substances circulating in the blood and lymph must, therefore, be able to keep these central parts alive. In other animals the substances present in the lymph or blood are only sufficient to keep the peripheral parts alive. Here, however, they even permit them to grow. We therefore see that the difference in the result is only of a quantitative character, growth taking place in both cases.

Further, we see distinctly the influence of pregnancy in producing almost identical changes in the original and in the

¹ In the guinea-pig the normal mammary gland has been successfully transplanted by Ribbert. One of these transplanted glands produced milk during the following pregnancy of the animal. Arch. f. Entwicklungsmechanik, Bd. vii.

transplanted pieces. Of interest in these experiments is the behavior of the mast cells. In the piece transplanted into the same animal the transplanted mast cells remained preserved. They undergo the same variations in their contents of granules as in the original tumor. Many of them seem to lose their granules, and to become indistinguishable from ordinary mononuclear cells, situated in the connective tissue. In the pieces transplanted into other animals, we find still a very small number of mast cells preserved, at a place where the gland cells have been destroyed. A larger number of them are still present where in the periphery of the transplanted piece the surrounding connective tissue advances. The large mass, however, of the mast cells transplanted into the male rat had a different fate; they were all filled with very large quantities of granules, a part of which had probably to be newly formed after transplantation.¹ All of these mast cells gradually disintegrate under the new conditions; only a small number of mast cells are still well preserved on February fourth. Frequently granules partially clump together. In others the granules are scattered into the surrounding tissue. At many places the cells have been absolutely dissolved, the granules only remaining in the connective tissue. February twenty-second all mast cells have here disappeared. These facts show that the mast cells perish simultaneously with the gland tissue; that they do not leave the place where they were situated at the time of transplantation. The increase in the number of granules in these mast cells after transplantation is perhaps to be explained by the storing-up of material derived from the gland cells during the process of necrobiosis of the gland. It has further been made probable through these observations that mast cells can lose their granules and become indistinguishable from small surrounding mononuclear cells. At other times perhaps new granules in such cells are formed.²

¹ A somewhat analogous fact is the observation of Unger, that during stagnation of milk in the human breast mast cells appeared. Virch. Arch., Bd. 151.

² On Mast Cells in the Mammary Gland: E. Coen, Beiträge z. Normalen u. Path. Anat. d. Milchdrüse. Ziegler's Beiträge, Bd. ii. Unger, das Colostrum, Virch. Arch., Bd. 151. Also Ehrlich und Lazarus. D. Anæmie. Nothnagel's Specielle Path. u. Therapie. Williams, Journ. of the Med. Sci., 1900. Harris, Phila. Med. Jour., 1900.

In conclusion it might be stated that, although in smaller animals like rats it may be difficult to secure animals with tumors for experimental investigations, it is easy to find them in larger animals, as, for instance, in cattle. With Dr. G. Jobson I showed¹ that in cattle one variety of carcinoma can be regularly found, namely, the carcinoma originating at the inner canthus of the eye, in the region of the caruncle. To this place all foreign bodies getting into the eye are carried. This is relatively the most frequent seat of carcinoma in cattle, although we have observed it since in various internal organs, and twice at the vulva.² Out of thirty-two cases more carefully examined, we found a metastasis present twenty times in the retromaxillary lymph glands. Just as in man carcinoma occurs mainly in older people, so usually only cattle above seven years of age are affected. As Behla and others found certain localities, where human carcinoma seemed to be endemic, so we found such a place in the case of carcinoma of cattle. I also observed different cases of multiple carcinoma in cattle. The analogy between human and bovine carcinoma is, therefore, very great, not only from an anatomical, but also from a clinical point of view, and experimental results obtained with tumors in cattle could be applied to carcinoma in man.

Summary.

1. Further successful transplantations of tumors found in white rats into animals of the same species are reported, especially a series of transplantations of the sarcomatous part of a mixed adenocarcinoma—sarcoma of the thyroid.
2. The reasons are stated why these new growths must be regarded as real sarcomata and not as granulomata.
3. The sarcomata found in different rats showed some structural differences based on differences in the cells, on

¹ L. Loeb and G. Jobson. On Carcinoma in Cattle. *Medicine*, April, 1900.

² My first experiments on transplantation of tumors were made in 1899 with cattle. I also injected filtered pressjuice of tumors into animals to test the presence of ferments in or between the cells which might cause the growth. The result of these experiments was negative, perhaps owing to the unfavorable conditions under which the experiments had to be carried on at that time.

differences in the formation of fibers, and in the degenerative processes which take place. These differences are present, although these sarcomata originated in the connective tissue of the same part of the body; they were reproduced throughout both series of transplantations which were carried through about forty and eight generations respectively.

4. Certain factors in the constitution of these tumors were constant and others variable. The bearing these facts have on the classification of certain sarcoma has been discussed. The possibility must be admitted that the relation of sarcoma cells to blood vessels might be dependent on nutritive and functional and not alone on genetic relations.

5. Further investigations make it certain that many peripheral sarcoma cells remain alive in the first few days after transplantation and that they mix with the growing connective tissue of the neighborhood. It is very probable that these peripheral sarcoma cells give rise to the tumors after transplantation.

6. The growing sarcoma has the power to penetrate into the central necrotic part of the transplanted piece, which becomes liquefied, probably under the influence of proteolytic ferments. Also necrotic tumor tissue around thrombotic blood vessels may become dissolved.

7. In the second series all attempts to produce tumors by transplantation of pieces or by injection of tumor fluid in white mice, guinea-pigs, rabbits, have been without result.

8. In both series of transplantations pieces of tumor which were infected at the time of the operation frequently gave rise to the growth of sarcomata. The actively growing tumor cells seemed to possess considerable power of resistance to bacterial toxins.

9. In the new series of experiments injection of cystic fluid or of a fluid obtained by mincing the tumor with addition of normal salt solution did not produce sarcomata. Negative results, therefore, which were obtained with fluid filtered through different varieties of filters cannot be regarded as indicating the character of the tumor-producing agency.

10. The question, if individual immunity exists, is discussed and it is shown that several complicated factors make it difficult to prove its existence. Of especial importance in this connection is the fact demonstrated by my former experiments that local conditions determine in some measure the fate of a tumor. Similar complicated circumstances exist with regard to the question, if in the course of transplantation a decrease in virulence takes place. In the problem of immunity two factors must be distinguished: first, immunity against transplanted sarcoma cells; and secondly, immunity against the tumor-producing agency connected in some yet unknown way with the tumor cells.

11. Local metastases and contact metastases were also formed in the second series of transplantation. It is probable that the rarity of hydropic cells in mytotic division in the cystic fluid in the second series of transplantations causes the contact metastases in the second series to be much rarer than in the first series.

12. In the second series of transplantations secondary nodules were frequently formed if ulceration of some part of the tumor had taken place.

13. The curve representing the growth of the transplanted pieces was similar in both series. In the case of the first cystic tumor as well as in the case of the mixed tumor the growth of the original tumor was slower than the growth usually observed in the transplanted pieces.

14. A series of experiments was undertaken to determine how long pieces cut out from tumors could be kept on ice and yet develop sarcomata after transplantation. Pieces kept on ice for one, two, and five days developed in each case several tumors. These experiments prove that the tumor-producing agency is not destroyed or even markedly attenuated after having been kept on ice for five days. The same holds probably good for the sarcoma cells themselves. By modifying this mode of experimenting, it may be expected that we shall get an insight also into other properties of the sarcoma-producing factor, even without having isolated it, in a similar way as ferments have been studied, although they have not yet been isolated.

15. Experiments on the influence of Röntgen-rays were begun. After seven exposures of ten minutes each the sarcoma cells continued to multiply by mitosis. Pieces transplanted from a tumor treated in this way developed sarcomata. In the center of the tumors degenerative softening was present.

16. The result of the transplantations of the adenoma of the mammary gland was different, according to the animal into which pieces were transplanted. Pieces transplanted into the same animal remained alive *in toto*, and underwent afterwards the same cycle of changes as that part of the original piece which was left back during the operation. Pieces transplanted into other rats became almost entirely necrotic. In one of these pieces, however, some peripheral parts of the glands remained alive for a few weeks, and did even grow by mitotic division.

17. In the original piece and in the piece transplanted into the same rat, pregnancy induced a large increase in the size of the tumor, especially in the glands, and probably also many mitoses could be seen in the glands and mitoses were also present in the connective tissue.

18. Mast cells present in the tumor were successfully transplanted. Their later fate depended upon the fate of the transplanted tumor. Where the glands remained alive, the mast cells also remained alive, and underwent similar variations in the number of their granules, as the mast cells of the original tumor. It is probable that some of the mast cells can lose all of their granules. In the pieces which became necrotic after transplantation, the peripheral mast cells remained alive, wherever the surrounding connective tissue advanced. In the central part, however, the mast cells formed large masses of granules, during the few weeks following the transplantation. Then they disintegrated, scattering their granules into the connective tissue. A few weeks later, when the gland tissue had disappeared, the mast cells also had disappeared by disintegration. They did not migrate from the central part, which became necrotic, into the surrounding healthy tissue.

EXPLANATION OF PLATE.

PLATE V.

FIG. 1. — Sarcomatous part of the original adenocarcino-sarcoma of the thyroid. Spindle cells. a, a₁. Mitoses. Obj., 8 mm., Oc. 4.

FIG. 2. — From another part of the same tumor. Shows the arrangement of the sarcoma cells around the blood vessels. a, a₁, a₂. b. Degrating parts of the tumor; only few nuclei have been left in the fibrous tissue. Obj., 16 mm. Oc. 1.

ON TUMOR TRANSPLANTATION AND INOCULATION.¹*(Preliminary Report.)*

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Mayet,² who for a number of years has been working along the line of artificial tumor production, in a recently published paper says that he has become convinced that there exists in cancerous neoplasms a soluble principle which, when injected into an organism, may produce a proliferation which one can describe as cancerous. This, however, would neither confirm nor exclude the parasitic theory. Mayet's experiments were made on white rats, rabbits, and dogs. In principle he excludes experimental transplantation of tumor tissue entirely, as being unfit to lead to a recognition of the true etiology of tumor formation, and he says with reference to it: "La greffe est une procédé peu probant pour l'étude de la pathogenie du cancer. Ce qu'on doit rechercher c'est l'introduction dans l'organisme du principe actif, parasite ou ferment, indépendamment des cellules néoplasine." In spite of this statement, Mayet has made some transplantation experiments in introducing pieces of human cancer tissue into subcutaneous pockets, or in rubbing up pieces of carcinoma with sterile bouillon, and injecting the resultant emulsion into the circulatory system or into the peritoneal cavity of animals. In most of his experiments Mayet, however, used a glycerine extract of human aseptically macerated cancer tissue, or a filtrate obtained by filtering through a Pasteur filter or an asbestos filter. Quite a number of the animals used in the experiments received

¹ Read by title, March 30, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, Ohio.

² Mayet. "Production du Cancer chez les Rats blancs par Introduction dans leurs Économies des Substances constituantes des Tumeurs malignes de l'Homme." *Gaz hebdom. de Medic. et de Chir.*, Jan. 19, 1902; No. 6, p. 64.

preliminary cantharidin injections, to produce a transitory albuminuria, and so to predispose the kidneys for the development of cancer nodules. Mayet concedes that his experiments with dogs and rabbits were negative, but in his experiments with fifty-three white rats he says that he *certainly* did four times produce "des lésions cancéreuses épithéliales," and in eight more cases *probably* similar lesions were produced, but not as unmistakably pronounced as in the four first mentioned cases. Mayet's alleged successful cases were those with glycerine extracts or filtrates of human carcinomatous tissues.

I think that one cannot read Mayet's descriptions critically without becoming convinced that he has not succeeded in producing a true carcinoma, in a single instance, or, as is claimed in one case, a true sarcomatous nodule, in a rat's liver caused by the injection of a filtrate from a human sarcoma.

I have myself for several years tried to produce a carcinoma or sarcoma in rabbits and guinea-pigs by injecting triturated and filtered human material, subcutaneously, intraperitoneally, and into the anterior chamber of the eye. I have never had any success in these experiments. Nodules were sometimes formed in the anterior chamber of the eye, but they soon again disappeared. Occasionally there were also found coccidium tumors in the livers of rabbits, but they had, of course, nothing at all to do with the injected human tumor material.¹

Loeb² last year reported his successful tumor transplantations. One of the male white rats kept in the Laboratory of the Polyclinic (and I believe a descendant of the white rat which became Loeb's original starting material) during the summer of 1901 developed a tumor of the neck in the region of the thyroid, which was shown to be a very vascular, cystic sarcoma; in fact, identical in every respect as to its histology with the rat tumor used in Loeb's successful transplantation experiments.

¹ In some of these experiments made four and five years ago surgical operations were made for me by Drs. M. L. Harris and Wm. H. Wilder.

² Loeb. On Transplantation of Tumors. The Journal of Medical Research, Vol. vi (New Ser., Vol. i), 1901, p. 28.

This material proved as successful in my hands as it had proven in Loeb's hands. Numerous transplantations were made for the purpose of obtaining for a longer period of time fresh tumor material, and to make from it cell-free preparations for inoculation experiments. Before reporting upon these inoculation experiments, a summary of the observations made in the transplantation experiments may be given. These latter were in themselves not the real object of the work, but they furnished results worth reporting. There were made seventy to eighty transplantations in all. The tumor material was always obtained and implanted with all aseptic precautions, and only in a very few instances did infections interfere with the experiments. Pieces of tumor were transplanted either subcutaneously or intraperitoneally. It may be stated that all tumors, even if non-infected from the start, frequently became infected when they had attained a large size, and had either led to necrosis of the overlying skin or to intraperitoneal adhesions. Such infected tumors were, however, never used in transplantations, and these were preferably practised from subcutaneous tumors, as long as they were still small (not larger than a good-sized hazelnut or a small walnut). The first transplantation was made from the male white rat, which had spontaneously developed the tumor, to three white rats. In two of the animals operated upon the tumors grew quite slowly. In one, a pregnant female where a piece had been implanted subcutaneously into the lower abdominal region, the tumor grew quite rapidly. This female gave birth to a litter of young ones, which she raised successfully in spite of the growing tumor. Pregnant females were subsequently repeatedly subjected to implantation of pieces of tumors. It was invariably found that when a piece was implanted subcutaneously into the abdominal region of a pregnant female, the tumor grew with unusual rapidity, attaining a large size in two or three weeks. About thirty to forty young ones were successfully raised from such females; not one of these young ones has spontaneously developed a tumor. Of the seventy to eighty transplantations made, all except fifteen

were successful. Of these fifteen, three cases can practically not be considered, because these rats died shortly after the operation from a peculiar accident. Four rats, after having been shaven, had been washed, not, as usual, with a bichloride solution, but with a five per cent carbolic acid solution. Directly after the operation these four animals went into convulsions; three died inside a few hours, and only one survived, and developed a rapidly-growing tumor. Once pieces of tumor from a white rat were implanted into ten white rats which had not been raised, like the rest of the rats operated upon, in the Laboratory, but had been sent from a distance and were furnished to me by Dr. L. Loeb. Not a single one of these rats developed a tumor. Five of them, which I kept, were operated upon a second time, but they have so far not yet developed any tumors, and are still under observation. The most marked example of resistance was furnished by a large white male. This animal was operated upon subcutaneously three times, at intervals of three to four weeks. All three implantations did not take. Finally, on December nineteenth, 1902, a large piece of a non-infected tumor was implanted intraperitoneally. Four weeks after this last implantation the animal was found dead one morning. A post-mortem showed neither tumor nor infection. Small cicatrices marked the places of implantation, but that was all; the internal organs showed no changes to the naked eye. The material from these organs has been fixed and embedded, but no microscopic examination has, as yet, been made. The sudden and unexpected death of this rat prevented its blood being used for an attempt at immunization as was intended. Aside from these failures, all implantations were successful; the rats used were of either sex, young and old, white, black, and gray, the latter, however, not being full-blooded gray wild rats, but half-breeds. Sometimes the implanted material led rapidly to the formation of a large tumor, so that one of the size of a large walnut or a small apple had been formed in from four to six weeks. At other times only small, hard, slowly-growing nodules formed. Pieces from such small tumors

formed very rapidly-growing tumors when transplanted again, and in place of the small nodules removed, a rapidly-growing tumor sometimes developed. In all operations, even where a thorough removal of the tumor had been made, a local recurrence took place, either speedily or slowly. I have never observed distant metastases, though I have seen subcutaneous tumors break into the peritoneal cavity. When left alone, the tumors always tend to sloughing and to local infection.

Histologically, the tumors consist of spindle or round cells, quite embryonal in type. The neoplasms are very vascular, form cystic spaces, and often, when extensive, contain large tracts of necrotic material, even long before an infection has taken place. In some cases, examined for this purpose, I have seen remnants of the implanted piece or pieces as a necrotic material, surrounded by young healthy tumor tissue, the latter showing no regressive but only progressive changes. The necrotic pieces implanted are found to contain numerous polynuclear leucocytes at their boundary. The presence of these I do not think to be due to an infection, but they are apparently attracted by chemotactic influences originating from the necrotic area. The implanted piece may perhaps be properly compared in its later appearance, as well as in its influence upon the surrounding tissue, to a non-infected anemic infarct. Non-infected to be understood, as far as known microorganisms, but not as far as possibly present unknown tumor organisms are concerned.

Inoculations made from non-infected tumors on the usual culture media were always negative.

To summarize, over fifty transplantations were successful, and these extend over eight tumor generations; counting the starting material as tumor generation No. 1, the first transplantations as tumor generation No. 2, and so forth. Most operations were made for me by Dr. V. Baccus, whose strict antisepsis almost invariably prevented any septic complications.

Attempts to transplant or inoculate the tumors to rabbits were negative. The blood of rabbits treated with tumor

material from rats in various ways is to be used in attempts at immunization.

Treatment of rats having tumors by exposing the latter to the Röntgen-rays has recently been begun. The exposures, lasting ten minutes, were kindly made for me by Dr. R. R. Campbell. He used a high vacuum Mueller's tube and a Scheidel's coil. Two rats have been treated. They had fairly large non-infected subcutaneous tumors. The skin over them was in good condition. In both, the skin over the tumor, under the influence of the exposures, became necrotic. In one rat the tumor after a few exposures became changed into one large cyst filled with a perfectly fluid material. After the fifth exposure (made on the sixth day from the beginning of the treatment) the whole tumor came away, leaving a clean surface. The rat, however, died shortly after this occurred, unfortunately while I was out of town. The second rat has now had eight exposures during ten days, and its tumor has become very soft likewise, and has markedly decreased in size.¹

INOCULATION EXPERIMENTS.

The experiments to produce a tumor from absolutely cell-free substances derived from these sarcomata, as far as I can report about them to-day, were the following:

Pieces of non-infected tumors removed with all aseptic precautions were dropped at once into a sterile mortar containing sterile quartz sand and silicon powder. The tissues were thoroughly ground up, and while this was done physiologic salt solution was added. The emulsion so prepared was then filtered through a Pasteur filter. From the filtrate a number of culture tubes were inoculated to show it to be free from known microorganisms. Several cubic centimeters of the filtrate were injected into the abdominal cavities of rats. Not a single one of the rats so treated developed a tumor, even when treated in this manner repeatedly. Some were killed and examined; the findings were absolutely nega-

¹ Since the above was written, further exposures to X-rays have been made and I have come to the conclusion that these rapidly growing sarcomata cannot be cured by the X-rays, though sometimes a temporary improvement may occur.

tive. One black female treated with the filtrate emaciated more and more, and one morning it was found dead. The post-mortem and a subsequent careful microscopic examination of all of the internal organs (except the central nervous system) failed to show either a tumor or the cause of death.

Two of the rats, repeatedly treated with the filtrate, have been under observation for six months. They are well and fat, and show no trace of any tumor or other affection.

While it formerly must have appeared an absolutely unpromising task to transplant any supposed infectious or parasitic affection by a tissue fluid filtered through a Pasteur filter, we now know from the work of Nicolle and Adil Bey, and others, that certain obviously infectious diseases, like "Peste Bovine," can be propagated by such a filtrate, and that its cause must be a living organism, too small to be seen even by the aid of our best optical instruments. It is also possible that a filter with larger pores than the one employed in the above experiments might lead to a better result.

In another series of experiments, undertaken to produce tumors from a fluid derived from tumors, but free from tumor cells, *collodion sacs* were employed.¹ I first implanted these sacs into small subcutaneous tumors, but this method proved a failure since the sacs invariably sloughed through the skin.

I then implanted them intraperitoneally, either with a piece of tumor, or into a tumor which had already been developing for some time. After the sacs have been left a variable time in the abdominal cavity, they are removed with particular aseptic precautions. They may be either found free in the peritoneal cavity, or they may be entirely surrounded by tumor tissue. Their contents, clear when implanted, are found to be more or less cloudy or hazy. These sacs were removed with sterile forceps. One point of their collodion surface is cleaned with sterile filter paper, and then touched with a moderately hot knife, to destroy any adhering cells. This procedure may burn a hole in the collodion, and the sac must be so held that the fluid contents cannot flow out. Next a fine sterile glass pipette is inserted, and the fluid con-

¹ These sacs were prepared by Messrs. Parke, Davis & Co.

tents are withdrawn. Part of the contents are at once injected into the peritoneal cavity of one or more rats ready for the operation. The rest of the fluid is dropped into culture tubes.

No cultures have developed in these experiments, nor have any of the rats so treated developed a tumor. No death occurred among the animals so treated.

I have also in these and other experiments considered the possibility that ultra-microscopic organisms, which might be the cause of malignant tumors, might be so strictly parasitic that they cannot exist outside of living cells. This conception may not be correct, but we must at least keep it in mind in experiments having for their ulterior object the discovery of a living cause of malignant tumor formation. I have for several years attempted to make use of living saccharomyces as a culture medium for supposed ultra-microscopic organisms, and I have also made use of them in these tumor inoculated experiments. Several kinds of yeast cells were used in connection with the collodion sacs, but without success. Perhaps better results may be obtained in using known types of yeast cells without the collodion sacs, to bring about a symbiosis between the unknown looked-for ultra-microscopic organism and the saccharomyces. But in the use of such methods one encounters great difficulties in keeping alive yeast cells, injected into animal tissues, against the combined hurtful influences of the high body temperature and the reaction of the living body cells.

I cannot to-day report upon experiments in this direction, but I can state that I have for years used methods involving the use of living yeast cells as a culture medium for supposedly existing ultra-microscopic tumor organisms. My results in these experiments have been by no means satisfactory or even promising, but I have always, in spite of innumerable failures, returned to these methods, well remembering Pasteur's words: "*Refaisons la même expérience, l'essential est de ne pas quitter le sujet.*"

DESCRIPTION OF PLATES.

PLATE VI.

- No. 1. Rat with subcutaneously implanted tumor; third generation.
No. 2. The same; fourth generation.
No. 3. The same; fifth generation.
No. 4. The same; seventh generation. These four photographs were taken from the living rats.

PLATE VII.

- No. 5. Frozen section through collodion sac, surrounded by tumor tissue. The sac had been dissected out of the tumor mass in which it had become embedded. (a) Collodion sac; (b) tumor tissue. Leitz Pantachr., 34 mm.; Spencer aplanatic eye-piece 1 inch.
No. 6. Section showing type of tumor tissue, spindle-cell sarcoma. Spencer, Prof., $\frac{1}{4}$ inch. Eye-piece as above.
No. 7. Section through implanted piece and surrounding newly-formed tissue. (a) Necrotic implanted piece. (b) Newly-formed tissue. Leitz Obj., No. 3. Eye-p., No. 3.
No. 8. Same as No. 7; more highly magnified. Spencer, Prof. $\frac{1}{4}$ inch. Aplanatic eye-piece, 1 inch.

PERSISTENCE OF VARIETIES OF THE BACILLUS DIPHThERiÆ AND OF DIPHThERIA-LIKE BACILLI.*

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The fact that there are many varieties of the diphtheria bacillus has been fully established.

But that such varieties are true sub-species with constant characteristics, one variety not readily if ever changing into another, has not been demonstrated. On the contrary, of late the idea seems to be gaining ground among some investigators that all of the various forms of diphtheria-like bacilli are the result of more or less transitory variations of the same species, and hence that the virulent forms are the result of a rapid adaptation to environment and consequent pathogenesis of the non-virulent forms both typical and atypical.

This question of the relationship of the specifically virulent diphtheria bacillus to non-virulent, diphtheria-like bacilli has been discussed since the descriptions of a bacillus called the pseudo-diphtheria bacillus were published by Löffler¹ in 1887 and von Hofmann-Wellenhof² in 1888. Löffler's prediction that still more bacilli resembling the true diphtheria bacillus would be found, just as many bacteria similar to the cholera spirillum had been reported, has certainly come true. Many forms under the name "pseudo-diphtheria bacillus" have been described, many under the name "xerosis," and many as non-virulent diphtheria bacilli. The descriptions of Löffler and von Hofmann and of those immediately following have left us in some doubt as to which organism was meant by the pseudo-diphtheria bacillus. Each observer was probably describing a different variety of diphtheria-like bacilli, different characteristics were noted, and hence the difficulty when

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trying to compare results. In Park and Beebe's³ work of 1895 a complete tabulation of the results of the studies on the so-called pseudo-diphtheria bacillus up to that time is given, and the reason for the confusion in which the descriptions left us is plainly shown.

These observers give the name "pseudo-diphtheria bacillus" to a group of organisms with certain definite cultural and morphological peculiarities, different from those of the diphtheria bacillus, and for this reason as well as from their further observations that such bacilli were not found in typical diphtheria and that no case of infection from them was noted, they consider them an entirely distinct species from the diphtheria bacillus. The typical non-virulent bacilli they consider diphtheria bacilli which have lost their virulence. This belief is based first upon their own observations, second upon those of Escherich and others that bacilli of different degrees of virulence were isolated from diphtheria cases, and third upon the well-known work of Roux and Yersin in which they state that they found diphtheria bacilli of all degrees of virulence up to their most virulent forms, that non-virulent bacilli were more frequent towards the end of the disease, that they had deprived virulent diphtheria bacilli of their virulence, and raised the virulence of slightly virulent bacilli, though they had not been able to give virulence to bacilli which had lost all virulence. Park and Beebe state that the typical non-virulent diphtheria bacilli are probably incapable of causing diphtheria, for the twenty-four cases in which they were found by them never developed any lesions, nor were they the origin of any case of diphtheria, so far as could be ascertained. The observations of Escherich seem to show that diphtheria bacilli have more stable properties than one is led to believe from the work of Roux and Yersin. He found that most bacilli from cases of diphtheria were fully virulent, that they retained their virulence throughout the course of the disease, that the same degree of virulence was possessed by bacilli from different cases which were apparently infected from the same source, and that the virulence was retained for a long time on

artificial culture media. He found only occasionally non-virulent typical forms which he regarded as diphtheria bacilli which had lost virulence, and the bacillus he describes as pseudo-diphtheria bacillus, and which is probably the same as that described by Park, he considered a distinct species on account of morphological and cultural differences.

Until 1896 no one had brought forward evidence to show that fully non-virulent forms could be made virulent. In this year Trump⁴ states that he converted a non-virulent bacillus which he said was similar to those described by Hofmann, Löffler, Escherich, and others as pseudo-diphtheria bacilli (though it produced acid), into one capable of killing guinea-pigs with all the symptoms of true diphtheria, by successive passages through guinea-pigs plus a non-fatal dose of diphtheria toxin. From the description of the bacillus it should be classed with the non-virulent forms of the diphtheria bacillus. Hewlett and Knight⁵ state (1897) that they changed a typical virulent diphtheria bacillus into a non-virulent bacillus of the pseudo type by heating for seventeen hours at 45° C. They only succeeded with one culture, though they tried others. They say also that they changed a pseudo-diphtheria bacillus, similar to Park's, into a typical virulent diphtheria bacillus by culture and passage through guinea-pigs. They obtained similar, but not such marked results with other cultures.

Richmond and Salter⁷ (1898) and Salter⁸ (1899) state that they have changed five pseudo-diphtheria bacilli into typical diphtheria bacilli specifically virulent for guinea-pigs by passage through a number of goldfinches, and that a substance produced by the pseudo-diphtheria bacillus in broth cultures unites with diphtheria toxin as Ehrlich's protoxoid does. Their conclusion is that there are diphtheria bacilli of every grade of virulence from the Hofmann's (their description agrees with Park's pseudo) or pseudo-diphtheria bacillus capable of killing only certain birds, up to those that kill certain of the rodents.

Peters⁶ distinguishes two morphological species of pathogenic diphtheria bacilli, each of which kept its special mor-

phological character for two years on artificial culture media, except that the short form became more like the "Hofmann's bacillus" and lost its virulence. He thinks that there is no proof that the Hofmann's bacillus is an attenuated form of the diphtheria bacillus.

Axenfeld⁹ was not able through long cultivation to change one form into another, therefore he considered such forms sub-species. Bergey¹⁰ found some non-virulent forms culturally and morphologically different from the virulent forms; he was not able to give virulence to these non-virulent forms, neither did he find that these latter gave immunity against the former; for these reasons he considers them distinct members of a large group of bacilli at the head of which stands the diphtheria bacillus.

In the work of Wesbrook, Wilson, and McDaniel¹¹ on "Varieties of *Bacillus Diphtheriæ*," the study is based upon the morphology of the individual bacillus found in smears of throat cultures and pure cultures. They give as a reason for the study of the individual bacillus that in "pure cultures in most instances, especially where they have been derived from typical clinical cases of diphtheria, it is the exception to get even a moderate degree of uniformity in the general shape, size, staining reactions, etc., of the individual bacilli; whilst to get complete uniformity is not to be hoped for," and therefore each culture is probably a mixture of several varieties having been derived from several parents. They make a provisional classification based upon the morphology of the individual bacilli, into three groups, called granular, barred, and solid, two of the groups into seven types and the other into five, two of the types corresponding with those in the other groups not having been seen. In a study of the types found in the smears from a series of direct cultures derived from clinical cases of diphtheria the authors state that there is generally a sequence of types in the variations which appear throughout the course of the disease, the granular types being the most predominating at the outset of the disease, and these giving place wholly or in part to the barred and solid types shortly before the disappearance of diphthe-

ria-like organisms. In one case the converse was seen. In a serial study of the pure cultures, while the types met with in the original culture remain the predominating types, yet "it appears that the granular types when predominating or unmixed with the barred or solid types in the original cultures have, however, a tendency, as in throat cultures taken in series, to become more and more mixed with or replaced by barred or solid forms altogether in the later examinations.

. . . Certain of these stocks, however, in which one of the solid types is the only diphtheria-like organism seen in the original throat culture, and in which the immediate succeeding cultures show only solid types, are found to contain later in the series some of the barred or granular types and seem to show a gradual transition from solid types through barred to granular types."

The inference drawn from this work is that the diphtheria bacillus may be rather easily, especially in the throat, converted into non-granular solidly staining forms of the "pseudo-diphtheria" type, and that the converse may occur, and that therefore all diphtheria-like bacilli must be considered a possible source of danger.

Cobbett¹² considers the pseudo-diphtheria bacillus as perfectly innocuous to man, but that the relation between the pseudo-diphtheria and the diphtheria bacillus remains undecided. He did not meet with bacilli of low virulence. He found a few non-virulent and the others were all highly virulent. No Hofmann's bacillus possessed any virulence. He thinks that the reason why the pseudo-diphtheria bacilli appear so infrequently during the acute stage is that they are overlooked then because one discovers the virulent bacilli so easily and does not trouble to look any more, and they are found more easily later because the diphtheria bacilli are disappearing and are hard to find; consequently a long and careful search is made, and the pseudo-diphtheria bacilli are seen for the first time.

Ohlmacher¹³ states "that most of the common characteristics of the diphtheria bacillus are unstable" and that the pseudo-diphtheria bacillus is but a modified variety of

Löffler's bacillus. He made his experiments in 1894, and reports them in January of the present year. He says that by one passage through a white rat he changed "a long granular or barred bacillus" into "a short, plump, uniformly staining rodlet, an example of the short, solid type of diphtheria bacillus of Westbrook, and of Gorham, which, by many authorities, is classed as the pseudo-diphtheria bacillus." From a second variety which was originally "a short, plump, solid rodlet with an occasional short granular example" he says he obtained, by one passage through a guinea-pig, "a long barred, or granular, 'typical' diphtheria bacillus." From a third variety of "the extreme atypical morphological type" he says he obtained, by one passage through a guinea-pig, "a long barred or granular 'atypical' diphtheria bacillus." Recently, the work of Wilson, Westbrook, and McDaniel has been corroborated by Gorham.¹⁴

All of this work (including the reports of observers not mentioned in this paper) in regard to the relationship of the different diphtheria-like bacilli to the true diphtheria bacillus may be summed up and tabulated as follows:

Statements in favor of the belief that one form may be changed readily into another.

1. — The morphological and cultural characteristics of all diphtheria-like organisms from pseudo to typical virulent forms have some points of resemblance.
2. — Diphtheria bacilli possess many grades of virulence from the fully virulent to the non-virulent.
3. — Non-virulent bacilli, both typical and non-typical, have been found more frequently in the convalescing stage of diphtheria than in the acute stage.

Statements opposed to this belief.

The morphological and cultural characteristics of some varieties have many points of difference.

Intermediate grades of virulence are rare.

There are other reasons than that of change of one form to another to account for this.

Statements in favor of the belief that one form may be changed readily into another. — (Continued).

4. — Non-virulent, atypical bacilli have been the only diphtheria-like organisms found in light anginas.
5. — A sequence of forms in the course of diphtheria and in successive generations of pure cultures, from granular through barred to solid forms, and the converse, has been observed.
6. — Solid forms, approaching the atypical non-virulent forms, have been found to be specifically virulent.
7. — The virulence of the diphtheria bacillus has been decreased artificially with a change in form and cultural characters, and slightly virulent diphtheria bacilli have been made more virulent.
8. — Non-virulent atypical bacilli have been changed to typical, specifically virulent diphtheria bacilli.
9. — Virulent typical diphtheria bacilli have been changed to solidly staining, non-virulent, diphtheria-like bacilli.
10. — The solidly staining non-virulent pseudo-form produces a substance which acts as Ehrlich's protoxide.

Statements opposed to this belief. — (Continued.)

Diphtheria bacilli and other organisms have also been found.

This remains to be further corroborated.

Among large numbers of virulent diphtheria no solid varieties have been found.

Artificial decrease of virulence of the diphtheria bacillus has not been made to a great extent, neither have slightly virulent bacilli been made highly virulent.

Non-virulent atypical bacilli have retained their characteristics on various artificial culture media under different conditions and in passage through animals.

Virulent typical diphtheria bacilli retain their characteristics on various artificial culture media under different conditions.

This remains to be corroborated.

The central idea in the statements of those who believe that diphtheria-like bacilli are simply transitory variations of the species *Bacillus diphtheriæ* is that both the diphtheria

bacillus and those bacilli which resemble them have many unstable properties, their form, their cultural characteristics, their pathogenicity all varying within a wide limit, so that one form may assume readily the properties of another form. The separatists, on the other hand, have found that certain forms possess such stable properties that one is not converted into another, and hence they regard them as distinct species.

In the work reported in this paper I shall attempt to show from studies of the diphtheria bacillus and of diphtheria-like bacilli extending irregularly over a period of seven years, that not only are there distinct species in this group, but that each species has distinct sub-species or varieties with characteristics which continue to persist under different conditions; thus varieties as well as species remain separate, and when grown under similar conditions the species show no tendency to become converted one into the other, while the varieties gradually change, approaching a common norm.

An outline of the work is as follows:

I. A study of the diphtheria and diphtheria-like bacilli found in a series of clinically typical diphtherias at the Hospital for Contagious Diseases.

(1.) Serial smears of cultures directly from throats and noses.

(2.) Pure cultures isolated from these cultures.

II. A study of the diphtheria and diphtheria-like bacilli found in healthy and diseased throats in a town during an epidemic of diphtheria.

(1.) Smears of cultures directly from throats.

(2.) Pure cultures isolated from these cultures.

III. A study of diphtheria and diphtheria-like bacilli found in sore throats during an epidemic of diphtheria at a home for destitute children.

(1.) Pure cultures.

IV. A study of pure cultures of diphtheria and diphtheria-like bacilli from sources other than those given above.

(1.) On various artificial culture media grown under various conditions.

- (2.) In living tissues of guinea-pigs, white rats, and goldfinches.
- (3.) In symbiosis with other bacteria.

I. A study of the diphtheria and diphtheria-like bacilli found in a series of clinically typical diphtheria at the Willard Parker Hospital. This study was undertaken in order to help solve the following questions:

First. Do the varieties of specifically virulent diphtheria bacilli change throughout the course of the disease?

Second. Are pseudo and non-virulent forms met more frequently at the end than at the beginning of the disease?

It may be well to say a word about what is meant by *variety* in this paper. The term is applied to a pure culture as a whole and not to individual bacilli, and by it is meant that certain pure cultures possess some persistent morphological and cultural characteristics so different from those of other pure culture that the culture has a distinct individuality. Such a pure culture may be composed of bacilli of many different forms, but since separate colonies from this culture produce cultures having the same distinct individuality as the original culture, each bacillus if it could be separated and made to grow by itself would presumably produce the same. This is contrary to Westbrook's idea that each "so-called pure" culture is a mixture of types, if he uses types as synonymous with variety, as he appears to do. That a pure culture is a mixture of "types" if types means forms is true, but that it is a mixture of varieties in the biological sense is not shown by these studies.

In the cases studied at the Willard Parker Hospital, cultures on Löffler's blood serum were made every other day from the nose and throat until the disappearance of all "suspicious" bacilli. Smears from twentyfour-hour growths were examined and the "types" found noted according to Westbrook, Wilson, and McDaniel's scheme. Pure cultures of the different varieties of diphtheria-like bacilli found in the first serum cultures were isolated, also from the tubes containing the last suspicious bacilli, each day's tubes contain-

ing twentyfour-hour culture having been kept in the ice-box after examination until the next cultures were examined.

In using Wesbrook's classification for indicating the "types" found in smears from the mixed cultures directly from the patient, it was often difficult and sometimes impossible to make the form fit. Though they have undoubtedly pictured the principal definite forms met in smears from cultures containing other bacteria, the variations in form of the diphtheria bacillus especially when grown with other bacteria are so numerous that qualifying words usually must be added to their letters. For instance, there are thick and thin, long and short Cs and Ds, granular, barred, and deeply staining Cs and Ds with intermediate and irregular forms innumerable.

All that can be said in tabulating the results according to Wesbrook's scheme is that the letters indicate the nearest approach to his types.

Examination of smears from primary blood serum cultures made from time to time throughout the disease and until the disappearance of diphtheria bacilli from the throats.

TYPES PRESENT (ACCORDING TO WESBROOK, ETC.).

Case.	No. Culture.	Throat.	Nose.
No. 1.	1	C—D, C ² , majority C.	No diphtheria-like bacilli.
	2	C—C ² " "	" " " "
	3	No diphtheria-like bacilli.	" " " "
	4	" " " "	" " " "
No. 2. Died the day after the sixth culture.	1	C, D, C ¹ , D ¹ , + and — granules.	C, C ¹ , D ¹ , D (C ¹ and D ¹ = + and — granules).
	2	C, D, C ² , D ¹ , C ¹ , + and — granules.	C, D, C ¹ , D ¹ (C ¹ and D ¹ = + and — granules).
	3	C, D, and strings of 3 and 4 C ¹ , D ¹ , + and — granules.	Same as throat.
	4	A, C, D, C ¹ , C ² , D ² , D ¹ , A ¹ , + and — gran.	About as throat.
	5	D ² , C, D, F ² , D ¹ (contaminated culture with large bacilli and many cocci).	D, C, D ² , D ¹ , C ² , C ¹ , + and — granules.
	6	C, D single and in strings, C ¹ , A, D ¹ .	C, C ¹ , D, single and in strings, D ¹ , D ² .

TYPES PRESENT. — *Continued.*

Case.	No. Culture.	Throat.	Nose.
No. 3.	1	D ² , C ² , A ² , C.	E ² (long), C, C ² , D ² , A, A ² .
	2	C ² , C, D, A.	C ² , E ² , D ² , C.
	3	C, C ² (C single and in strings of 2 and 3), D.	C, C ² (few strings of C), D.
	4	C, D, E, C ¹ , A.	D, E ² .
	5	C, C ¹ , D, E.	C, E ² , D, C ² .
	6	C, D, many in 2 and 3 strings, C ² , D ² , A, F, few with irregular granules, A ¹ + granules.	No diphtheria-like bacilli except E ² .
	7	No diphtheria-like bacilli.	No diphtheria-like bacilli except E ² .
No. 4.	1	C ² (short), occasional short bacillus with irregular granules.	E ² (long) and same bacillus as in throat, few.
	2, 3, 4	Occasional short irregular bacillus with irregular granules.	E ² (long and thick), occasional short thick bacilli with irregular granules.
	5	Occasional C ² , C ¹ , C.	D ¹ , E ² , B ² .
	6	Few C ¹ , C, D ² .	B ² , E ² , D ² , occasional A ² .
	7	C, C ² , D, D ² , A (few).	D, D ² , E ² , C (few).
	8	No diphtheria-like bacilli.	No diphtheria-like bacilli.
No. 5.	1	C ¹ , D ¹ , + and — granules, coccus forms, C, D, C ² , D ² .	C, D, C ¹ , D ¹ , coccus forms, + and — granules.
	2	C ² , C ¹ , D ¹ , with granules, D, C.	C ¹ , D ¹ , + and — granules, C ² , A ² , C, D, A.
	3	C ¹ , D ¹ + and — granules, C, D, in strings, A.	C ¹ , D ² , + and — granules, C, D, in strings, A.
	4	C ¹ , D ¹ , C.	D, C, C ¹ , D ¹ , A, D ² .
	5	C, D, C ¹ , A.	C ¹ , C, D, D ¹ .
	6	C (in 2 and 3), A, C ² , D ² , D, F, C ¹ (few).	C, C ² , A, D, F, C ¹ , few.
	7	No diphtheria-like bacilli.	C, D ¹ , D (few).
	8	" " " "	No diphtheria-like bacilli.
No. 6.	1	C ² , C.	E ² , E ¹ .
	2	No diphtheria-like bacilli.	E ² , D ¹ .
	3	" " " "	E ² .
No. 7.	1	C, D, A, D ² (3 and 4 in strings of C and D).	No diphtheria-like bacilli.
	2	D, D ² , C (scarlet fever? removed from ward).	" " " "
No. 8.	1	C, D, C ² .	E ² , D ¹ , C ² .
	2	C, C ¹ , C ² , D ¹ , D ² , A.	E ² , C ² , E ¹ .
	3	C, A, C ¹ , C ² .	E ² .
	4	D occasional (contaminated culture).	E ² .
	5	D ² , D ¹ , E ² .	E ² , E ¹ .
	6	C, D, D ¹ .	E ² .
	7	C, D, D ² , C ¹ .	E ² .

TYPES PRESENT. — *Continued.*

Case.	No. Culture.	Throat.	Nose.
No. 8.	8	C, D, A, C ¹ .	E ² .
	9	C, C ² , D, D ² , A.	E ² .
	10	C ² , C, D ² , D, few.	E ² , occasional.
	11	No diphtheria-like bacilli.	E ² , " "
No. 9.	1	C, D, E.	E ² , occasional C and E ¹ .
	2	C, D, C ² , D ² , C ¹ .	E ² .
	3	C ¹ (darkly stained), C, D.	E ² .
	4	Contaminated.	E ² .
	5	No diphtheria-like bacilli.	E ² .
No. 10.	1	C, D, E, A.	No diphtheria-like bacilli.
	2	No diphtheria-like bacilli.	" " " "
	3	" " " "	" " " "

Case No.	Culture No.	Pure cultures obtained from the W. P. H. cases above recorded.	
		(a.) Throat.	(b.) Nose.
No. 1.	1	C, D — principally on serum. Decidedly spreading colonies on agar and short bacilli. Rapid and abundant growth in broth with formation of decided pellicle within 24 hours. $\frac{1}{8}$ c.c. broth culture = death in 5 days of a 240 grm. guinea-pig.	
	2	Same as first culture.	
No. 2.	1	C ¹ , D ¹ , C, D and many small forms between D and E, though more irregular on serum. Small, non-spreading colonies on agar, many coccus forms with few larger thick bacilli and occasionally clubbed ends. Moderate growth in broth with formation of delicate pellicle after 3-4 days. $\frac{1}{8}$ c.c. ascitic broth culture = death in 4 days of a 230 grm. guinea-pig.	Same as throat culture.
	5	Same as first culture (isolated by the ascitic broth method).	Same as throat culture.
No. 3.	1	C, C ¹ , D — principally on serum. Moderately spreading colonies on agar composed of moderately thick bacilli with few segments. Good growth in broth with moderate pellicle in 24 hours. $\frac{1}{8}$ c.c. ascitic broth culture = death in 6 days of a 235 grm. guinea-pig.	Same as throat culture; also culture of solidly-staining pseudo-diphtheria bacillus (E ²).

TYPES PRESENT. — *Continued.*

Case No.	Culture No.	Pure cultures obtained from the W. P. H. cases above recorded.	
		(a.) Throat.	(b.) Nose.
No. 4.	6	Same as first culture.	5. Same as first culture.
	1	C, A, A ¹ , D — principally on serum. Non-spreading colonies on agar composed of thick bacilli with few segments. Slight growth in broth with no pellicle. $\frac{1}{8}$ c.c. ascitic broth culture = death in 4 days of 245 grm. guinea-pig.	Same as throat culture; also culture of solidly-staining pseudo-diphtheria bacillus (E ²).
	7	Same as first culture.	Same as first culture.
No. 5.	1	C, C ¹ , D ¹ + and granules irregular and large on serum. Non-spreading colonies on agar consisting of thick long bacilli with many segments. Slight growth in broth, slight pellicle. $\frac{1}{8}$ c.c. ascitic broth culture = death in 5 days of 245 grm. guinea-pig.	Same as throat culture.
	6	Same as first culture.	7. Same as first culture.
No. 6.	1	Similar to culture from No. 1.	E ¹ A regularly barred bacillus with delicate growth on agar and in broth-producing acid. 1 c.c. = death in guinea pig not controlled by antitoxin.
No. 7.	1	C, D, C ¹ , D ¹ on serum. Slightly spreading colonies on agar with short bacilli and large granules. Broth, good growth and moderate pellicle in 24 hours. $\frac{1}{8}$ c.c. ascitic broth culture = death in 4 days of 250 grm. guinea-pig.	
	2	Same as first culture.	
No. 8.	1	C, C ¹ , D similar to culture from No. 3.	Solidly staining pseudo (E ²).
	5	Same, no pseudos.	Solidly staining pseudo (E ²).
	10	Same, no pseudos.	Solidly staining pseudo (E ²).
No. 9.	1	C, A, C ¹ , D singly and in strings, on serum non-spreading colonies on agar with long segmented bacilli with Indian-clubbed ends, slight growth in broth with no pellicle. $\frac{1}{8}$ c.c. ascitic broth culture = death in	Solidly staining pseudo (E ²).
	3	Same as first culture.	Solidly staining pseudo (E ²).
No. 10.	1	Similar to culture from No. 3.	

In studying these cases from the letters we see that different "types" appear irregularly throughout the course of the disease, but no sequence can be observed. When pure cultures were isolated from a tube containing these different "types" it was found that exactly the same variety of bacillus and only this variety was obtained as from the earlier culture, showing that the new forms, which one finds in serial mixed cultures, are due, at least in part, to the influence of the other bacteria, and it is only when we study a pure culture that we can obtain a true idea of the variety to which the individual bacillus belongs.

In this series of cases no pseudo-forms appeared in the throats throughout the course of the disease that were not there at the beginning. Two of the cases had the solidly staining pseudo-diphtheria bacilli (type E^2) in the nose as the only diphtheria-like bacilli present there throughout the disease; one had this same variety as well as the typical diphtheria bacillus, and a fourth had a segmented pseudo-form (types E^1 and E^2); but in none of these cases did these pseudo-forms appear in the throat cultures, though they may have been present in the throat in small numbers. In regard to the appearance of solidly staining pseudo-forms towards the end of diphtheria I would say further that in an examination of hundreds of control smears made in the routine work for pronouncing diphtheria cases at the Willard Parker Hospital free from diphtheria bacilli, only occasionally were pseudo-forms observed, and in all of the cases where the typical bacilli persisted for some time and were isolated two and sometimes three times to test their virulence, the same variety continued to be present unmixed with new atypical forms.

In this first series of cases the typical diphtheria bacilli from the original nose cultures showed according to the letters slight differences from those from the throats of the same cases; pure cultures, however, showed that the same variety was in both nose and throat. Two cases, cultures from which were sent to the laboratory at this time, may be of interest in this connection. The first was a throat

case which had also a vaginal discharge. The same variety of virulent diphtheria bacilli was isolated from cultures from each locality. The second case had throat symptoms and a membrane on a finger wound. In this instance, too, the same variety was isolated from both cultures.

The pure cultures isolated from all of these cases were obtained in two ways: first by making agar plates directly from the original serum tubes, and second by the ascitic broth method. From the different agar plates made by these methods all the varieties of diphtheria-like colonies were planted on serum. If only one variety was apparent, cultures were made from at least three different colonies of this variety from each plate. That one variety only of the specifically virulent diphtheria bacillus was obtained from each case by these methods does not exclude the possibility of other varieties being present. It is quite possible that in a longer study of the individual colonies more than one distinct variety might be met with in the same case, but it is certain that in these cases the one variety isolated was the greatly predominating one throughout the disease and the probabilities are that it was the only one.

Some of the pure cultures (Nos. 1 and 4 from the throats and No. 6 from the nose) had such distinct characteristics that their recognition was especially easy and there could have been no question, even to the most superficial observer, in regard to their maintaining the same type throughout the course of the disease. In some of the other cultures the characteristics were not so peculiar, but they were not more or less peculiar at the end of the disease.

All of the pure cultures were kept at 36° C. on agar and transplanted every two to four weeks for five months, and two of the pseudo variety were grown on serum and in broth. At the end of that time when transplanted upon other culture media their individual characteristics were unchanged.

The results obtained from this serial study of smears and cultures from diphtheria cases may be summarized as follows: 1. In a series of ten cases of clinically typical diphtheria only one variety of the specifically virulent diphtheria

bacillus was obtained from the throat of each case throughout the course of the disease and until suspicious bacilli disappeared. 2. From different parts of the same patient only one variety of specifically virulent diphtheria bacillus was obtained. 3. Pseudo varieties were found no more frequently at the end than at the beginning of the disease. 4. Pure cultures continued to show the same characteristics for many culture generations.

We infer from these observations that specifically virulent diphtheria bacilli do not change readily, if ever, into any form of non-virulent diphtheria-like bacilli in throats or noses of people during an attack of diphtheria. The cases studied serially are few, it is true, but when we remember that most of them were not severe, and that in light cases especially we might expect to find changes in the type of bacillus approaching atypical non-virulent forms if such changes occurred with any frequency, this inference seems a fair one.

II. In the second group of cases studied in this connection, cultures were made principally from healthy throats. In a small town, during an epidemic of diphtheria, cultures were made from the throats of eighteen dairymen, fifty school-children, and forty-five children in a foundling home. All of the typical bacilli found were isolated and most of the atypical forms. The results from original smears and pure cultures are tabulated (using Wesbrook's letters) as follows:

TYPES PRESENT (ACCORDING TO WESBROOK, ETC.)		Variety of culture isolated.	Virulence on guinea-pigs.
Culture from throats of 50 school-children in Montclair, N.J.	No. 5. — E ² , D ² .	Solidly staining pseudo E ² and occasional D ¹ .	Non-vir.
	No. 48. — A, A ² , B, C, B ² , atypical.	Atypical B, A, C, B ² .	Non-vir.
	No. 4. — E ² .	E ² .	Non-vir.
	" 7. — D, E, very short.	E, D, short.	" "
Cultures from throats of 45 children in Foundling Home, Montclair, N.J.	" 9. — A, A ² , B, C, B ² , atypical.	Similar.	" "
	" 10. — E ² .	E ² .	" "
	" 15. — E ² .	E ² , E ¹ .	" "
	" 16. — E ² .	E ² .	" "
	" 17. — F, E ² , E ² .	{ 1, C, D, C ¹ . 2, E, D, C ¹ . E ² .	" "
	" 18. — E ² .	E ² , E ¹ .	" "
	" 23. — E ² , long.	E ² , long.	" "
	" 25. — C, D, D ² , C ² .	C ¹ , C, A, D.	" "
	" 26. — C, D, E.	{ 1, E, E ² , D. 2, A, C, D. E ² .	" "
	" 30. — E ² , C ² .	Similar.	" "
	" 31. — E ² , C, F, B, B ² , atypical.	E ² .	" "
	" 32. — E ² .	C, A, D.	" "
	" 33. — E ² , C, D.	C, A, D.	Virulent.
	" 38. — D, C.	C, D.	" "
	" 40. — C, D, A, F.	E ² .	Non-vir.
	" 44. — E ² .		
Culture from dairy, 18 throats.	No. 1. — E ² , D ² , C ² .	E ² .	Non-vir.
	" 2. — C ² , D, C, D ² , D ¹ , C ¹ .	D, C, C ¹ .	Virulent.
	" 3. — C, D, C, D ² , D ¹ , C ¹ .	D, C, C ¹ .	" "
	" 6. — E ² , D ² , C ² .	E ² .	Non-vir.
	" 8. — E ² , C ² .	E ² .	" "
	" 17. — B ² , B ¹ , C, atypical.	Similar atypical.	" "
	" 18. — C ² , D, C, D ² , D ¹ , C ¹ .	D, C, C.	Virulent.

The method employed for isolating the pure cultures was the same as that already described.

No case of diphtheria occurred among the school-children or in the home before the epidemic or up to three months later in the home, and yet these later cases contained an unusually large percentage of morphological typical diphtheria bacilli and of atypical bacilli. In some of the cases more than one variety of the morphologically typical non-virulent diphtheria bacilli were found in the same throat, ac-

accompanied, in one instance, by a typical virulent variety and in two others by the solidly staining pseudo variety (type E³). One reason why so many different varieties may be found in a normal throat is that no one is apt to overgrow the other, as is the case where one variety is pathogenic for a throat. Cultures Nos. 48, 31, and 9 (all the same variety) had peculiar characteristics: they were long, slender bacilli with very distinct granules and on agar plates grew in characteristic colonies. The very short granular varieties (Nos. 17 and 26) had also a distinct individuality. All of these cultures grown on serum and agar and transplanted every two to four weeks for six months and some for one year retained the characteristics of the original cultures. The non-virulent typical and atypical forms grown on serum were at first transplanted every week. After three months, inoculations into guinea-pigs showed, as did the original cultures, no pathogenicity for these animals. The virulent forms, however, retained their virulence.

The results noted in this group of cases may be summarized as follows:

1. In normal throats different varieties of diphtheria-like bacilli may be found in the same throat.
2. Many throats in a single institution may contain many diphtheria-like bacilli with no case of diphtheria resulting.
3. There are many distinct varieties of diphtheria-like bacilli, all of which in serial pure cultures retain the characteristics of the original culture.

The deduction from this set of observations is that non-virulent non-typical forms do not change readily, if at all, into virulent forms in healthy throats.

III. The third group of cultures studied came from a home for destitute children during an epidemic of diphtheria. A death occurred from diphtheria. Another followed in two weeks. No record was kept of the variety of diphtheria bacillus found in these two cases. A few days after the second death a boy who had slept in the same room with this patient at the beginning of the attack developed an

ulcerated throat. Cultures were made from his throat and from the throats of four other children who had very slight anginas. From the ulcerated throat one marked variety of virulent diphtheria bacillus was obtained and no other diphtheria-like organisms seemed to be present. From one of the light anginas an entirely different variety of morphologically typical diphtheria bacilli was isolated which was non-virulent for guinea-pigs. One of the other cultures contained pseudo-diphtheria bacilli (type E³) and the other had no diphtheria-like organisms. The cases with the two morphologically typical varieties were kept together. The two varieties persisted in the two throats for some time, the non-virulent one longer than the virulent, until finally both disappeared without change of type. The throat containing the non-virulent variety became immediately well. In the meanwhile other cases of ulcerated throats developed, all containing the same variety of virulent bacilli as that isolated from the first case. Cultures were taken from most of the children; some of the throats contained only pseudo-forms, but none of these developed diphtheria. One case of decided sore throat (not ulcerated) had, besides the virulent variety similar to the first, a pseudo-form (type E³), and a variety growing like a streptothrix in broth and on agar, but containing on serum as predominant bacilli forms approaching types D³ and D. Both the pseudo and the streptothrix-like varieties were non-virulent for guinea-pigs. The case containing the non-virulent morphologically typical bacilli had an ulcerated throat later, and cultures from it contained the same variety of virulent bacilli as that obtained from all the other cases.

These cases seem to be a very marked example of the spread of one variety of diphtheria bacillus among a number of people living together. These children all attended school and played together. There is no reason why other varieties should not have been present, except that they did not happen to be in that neighborhood at that time; also, no new children were brought into the home during the epidemic. These cases also explain why some of the most virulent

organisms are found in mild cases — the retention of virulence by the organism and the slight susceptibility of the patient. The children were given antitoxin and the virulent bacilli quickly disappeared from the throats. The one virulent variety isolated from all these cases is a very interesting one on account of its marked peculiarities. These are its many large coccus forms when grown on agar, (see Plate VIII., Fig. 1,) its very delicate colonies macroscopically on agar, its very scanty growth in broth, and the formation of large Indian-clubbed ends when grown on agar at room temperature.

This variety has been grown on agar, Löffler's serum, and in broth at different temperatures since its isolation on November twentieth, 1901, but no change in morphology or virulence has occurred. The summary of the study of this group of cases is as follows:

1. In cases of diphtheria apparently contracted from the same source the same variety of virulent diphtheria bacillus was found in each case.
2. This variety remained the only variety of virulent diphtheria bacilli found until its disappearance.
3. No gradations in pseudo types were found. The same type found in the first examination continued to be present in later ones.
4. Pure cultures continued to show characteristics similar to the original cultures.

The inference from these observations is that not only does a variety of the diphtheria bacillus retain its characteristics for some time in the same throat, but it may be transferred to other throats without losing its individuality.

IV. FINAL GROUP. — In the study of the pure cultures of diphtheria and diphtheria-like bacilli from sources other than those given above, the following points have been noted:

1. Original cultures:
 1. Their morphological and cultural characteristics on various artificial culture media.
 2. Their toxicity in liquid media.
 3. Their virulence from solid media.

2. Later culture generations:
 1. Persistence of original characteristics under the following conditions:
 1. On various artificial culture media.
 - a.* At different temperatures.
 - b.* Transplanted at different times.
 2. In living tissues of guinea-pigs, white rats, and goldfinches.
 3. In symbiosis with other bacteria.

During the past seven years about two hundred cultures of virulent diphtheria bacilli, ten cultures of non-virulent morphologically typical diphtheria bacilli, and fifty or more cultures of diphtheria-like bacilli have been isolated at various times. No attempt has been made to group the diphtheria-like bacilli, but among the specifically virulent diphtheria bacilli two broad groups have been recognized in which those varieties having the distinct characteristics of the one group differ widely from those having the distinct characteristics of the other, while between these extremes there are many gradations in type. So many gradations are there that almost every culture from a different source may be said to possess individual characteristics, though there are such slight differences between some of them that they may be classed as one variety.

To the first group belong the segmented varieties and to the second the non-segmented varieties.

The segmented and non-segmented varieties correspond respectively to Westbrook's barred and granular types.

When first isolated the individual characteristics of some of the cultures were very marked. It was found that some of the segmented varieties showed immense Indian-clubbed ends and many segments on agar, and that their growth in broth was extremely scanty. Such broth cultures gave very little reaction in guinea-pigs except in large amounts, though the bacilli themselves when inoculated from serum cultures were decidedly virulent. In ascitic broth, however, where they grew rapidly and abundantly, they showed a high degree of toxicity. After ascitic broth was used to test tox-

icity, all of the specifically virulent diphtheria bacilli, — about one hundred cultures, — segmented and non-segmented varieties, were found to be highly toxic for guinea-pigs. The largest dose of a two to six day culture in ascitic broth which was required to produce death in this animal was one-fiftieth cubic centimeter, the average dose being one one-hundredth cubic centimeter. The dose of a decanted culture containing one-half of one per cent carbolic acid was the same. These observations agree with those of Escherich and Cobbett, who found no decided grades in virulence among the diphtheria bacilli isolated by them. So far all of the non-virulent morphologically typical diphtheria bacilli have been non-segmented varieties.

From the pure cultures of virulent diphtheria bacilli ten of the most distinctive, five segmented and five non-segmented varieties, have been more closely studied. They have been grown at about 35° C. on agar, broth, and Löffler's serum transplanted at different times. One series, grown on agar and broth, was transplanted every two to six weeks. Another series grown on serum and agar was transplanted every one to three days, one in broth every day, every two to three days, and every week. For months two of the cultures were grown at 40° C., transplanted every week. Six were grown at 43°–45° C. alternating with 35° C. These six were also grown at room temperature and transplanted every two weeks, three were grown on hard-boiled eggs for eight culture generations, transplanted every week. Among these ten, four cultures are selected for detailed description as showing the greatest differences of one variety from another. The first two are non-segmented, and the second two, segmented varieties.

Number Eight (Plate VIII., Fig. 2) is the oldest culture studied. It was isolated in the summer of 1895 and the stock culture has been carried on in broth, re-inoculated from the pellicle every two to four days at first and for the past year every day.

When first isolated its characteristics were its moderately long cylindrical form on blood serum, its rapid and spread-

ing growth on agar with the production of short forms, its rapid growth in broth with the formation of a heavy pellicle and no clouding of the broth. Its virulence in broth averaged one two-hundredths cubic centimeters. The bacillus has now the same characteristics except that it has longer and more slender forms on serum and its granules are very small. After growing on agar for one year at 35° C. and being transplanted every two to six weeks, its characteristics remain the same. Grown on broth for a year and being planted every two to six weeks instead of every day, its characteristics remain the same, with the exception of an apparent slight loss of virulence.

Number Fifty-six (Plate VIII., Fig. 3) isolated February thirteenth, 1901. Its principal characteristics immediately after isolation were its short growth, moderately spreading colonies, and heavy pellicle on broth. Its average virulence is one-seventy-fifth cubic centimeter in broth. There has been practically no change in this variety after growth in broth and on agar and serum with varying reinoculations up to the present time. At one time the serum and agar cultures became contaminated with a streptothrix which was grown with it for some time, but no change was seen in the bacillus. Its short, thick forms and large granules present a marked contrast to number eight.

Number Thirty-one (Plate VIII., Fig. 4) isolated on the tenth of January, 1901. Its marked characteristics at first were its long, thick Indian-clubbed ends with irregular granules on serum; its much larger forms, especially the long, thick Indian-clubbed ends with irregular granules on agar; on agar too the growth is delicate, the colonies non-spreading and coarsely granular; in broth its slight growth in the bottom of the tube with no clouding and no pellicle. From serum to serum fewer Indian-clubbed ends appear, but when transplanted to agar from this medium the same characteristics develop. From agar to agar, re-inoculating every day, there are also fewer of the very large forms, but when transplanted to other media again the earlier characteristics appear. In

broth as in agar the growth becomes gradually more abundant and finally a pellicle is formed, but when transplanted to other media the original characteristics appear. Virulence in broth originally was very slight, but in ascitic broth one-seventy-fifth centimeter proved fatal. The average virulence remains the same.

Number Fifty-seven (Plate VIII., Fig. 5) isolated February thirteenth, 1901. Its principal characteristics at first were its long segmented form and small irregular granules on serum, its thick form with many segments and long Indian-clubbed ends on agar, also its delicate growth, small, non-spreading, coarsely granular colonies; its slight growth in broth with no pellicle and only a very slight finely granular growth in the bottom of the tube. From serum to serum the forms have become shorter and not so segmented. From agar to agar transplanted every day the morphology has changed decidedly, the bacilli are shorter and slender with tiny granules. The culture transplanted on agar, and in broth every two to six weeks, has retained its original characteristics except that the growth is more abundant. When the changed culture, however, is allowed to remain from two to six weeks again before transplanting the original morphological characteristics reappear.

This culture and number thirty-one grown on the hard-boiled yolk of hens' eggs for some time showed distinct morphological changes. The bacilli were all exceedingly small with comparatively large granules; in fact, they appeared almost like diplococci, the two granules being so close together. When grown on the other media again they assumed their original characteristics.

These four varieties after being in the body of an immune host (white rat) for forty-eight hours showed no morphologic changes. Grown at room temperature on agar and serum every two weeks, they show larger, more irregular forms than at 35° C. At 43° C. they show smaller forms than at 35° C., but in both instances growing at 35° C. again restores the original forms.

Though some of these cultures have changed on some of

the media, each one has changed in its own way, and each culture still has its distinct individuality. After many culture generations, especially when transplanted at short intervals, the different varieties tend to approach each other or rather to run in lines parallel with a common norm, which seems to be a medium-sized, non-segmented bacillus producing granules in early cultures on serum and growing well on all of the ordinary culture media. The non-virulent morphologically typical bacilli must be classed with the virulent varieties as one species, though there is little doubt that more minute study would show distinct species in this group. The atypical pseudo-forms, however, which show no tendency to approach the norm of the typical forms, must be classed as distinct species. All of the pseudo and non-virulent morphologically typical varieties when inoculated into the peritoneum of guinea-pigs in immense doses cause death. Attempts have been made to give more virulence to some of these varieties by successive peritoneal inoculations, but in no instance has any increase of virulence been noted or decided change in morphological or cultural characteristics. Two of the non-virulent, morphologically typical varieties have also been grown in symbiosis with virulent streptococci in broth for ninety culture generations transplanted every three to four days, but when separated no change in virulence or other characteristics was noted. Two other varieties of non-virulent morphologically typical bacilli have been inoculated into goldfinches with no result. In large doses they appear to be perfectly innocuous to these birds as well as do four varieties of pseudo bacilli, contrary to the results of Richmond and Salter.

Since there are so many different forms or varieties of diphtheria-like bacilli it is quite possible that some of them are so nearly related to the diphtheria bacillus that under certain conditions they readily develop its characteristics. This seems to be the only way to explain the apparent discrepancies in the results obtained by different observers. Such closely related varieties, however, do not appear to exist about New York City and its vicinity at the present time.

So we may safely say that as many non-virulent diphtheria-like organisms retain their characteristics, under various artificial and natural conditions, they may be regarded from a public health standpoint as harmless. These studies seem to demonstrate that the morphologically typical diphtheria bacillus is a distinct species from the atypical diphtheria-like bacilli and so-called pseudo forms, and that it has many true morphologic varieties or sub-species which while showing transitory ontogenic variations due to change in environment and life habit, have more or less persistent phylogenetic characteristics which reappear when the organism is placed in a previous environment.

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DESCRIPTION OF PLATE.

PLATE VIII.

FIG. 1. *B. Diphtheriæ*, No. 8. Twenty-four hours Löffler's serum culture. Long, slender, non-segmented forms. Very small forms on agar. Seven years on artificial culture media. X 1,410 diameters.

FIG. 2. *B. Diphtheriæ*, No. 31. Forty-eight hours agar culture. Thick medium clubbed rods and moderate number of segments. One year on artificial culture media. X 1,410 diameters.

FIG. 3. *B. Diphtheriæ*, No. 56. Twenty-four hours Löffler's serum mixture culture. Short, thick, non-segmented forms. Very small forms on agar. One year on artificial culture media. X 1,410 diameters.

FIG. 4. *B. Diphtheriæ*, No. 57. Forty-eight hours agar culture. Many segments; long, medium clubbed ends. One year on artificial media. X 1,410 diameters.

FIG. 5. *B. Diphtheriæ*, S. Twenty-four hours agar culture. Coccus forms. Segmented granular forms on Löffler's serum. Only variety found; cases of diphtheria at Children's Home. X 1,410 diameters.

ON THE GROWTH OF EPITHELIUM IN AGAR AND BLOOD-SERUM IN THE LIVING BODY.¹

LEO LOEB.

In a former paper ² I described the method by which I aimed to find means, first, to observe the growth of different tissues like epithelium separate from other growing tissues; secondly, to subject regenerating, isolated tissues to changes in the chemical composition of the surrounding medium and to observe the influence of this change of conditions on the growth. I also gave some results of these experiments so far as they were connected with the problem of regeneration. I now want to show that in epithelium growing in bloodserum in a guinea-pig certain features are produced of interest to the interpretation of some structural peculiarities found in carcinoma. Above all, it must be stated in this connection that epithelium under these experimental conditions shows only a limited growth, and that it never develops a carcinoma. It is not the tension of the surrounding epithelium which prevents the epithelial cells from multiplying rapidly and from growing apparently indefinitely, as it occurs in carcinoma. It is not the connective tissue beneath which resists the indefinite growth of the epithelium. For this growth to take place, some special chemical or physico-chemical conditions must be present. Under such experimental conditions as given here, however, irregularities of growth appear which bear some analogy to the growth of epithelial tissues in malignant tumors. Epithelium growing in bloodserum usually does not produce keratohyalin, for the production of which a close union with the connective tissues seems to be essential. This change in the structure is entirely due to the

¹ Demonstrated March 28, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, in Cleveland, Ohio.

² On the Growth of Epithelium. The Journal of the Amer. Medical Association, Oct. 19, 1901. A fuller report appeared in the Archiv. f. Entwicklungsmechanik, Vol. 13, where a detailed account of the method used is given.

conditions under which the growth is taking place. It is quite likely that all those changes in growing tumor cells, which have been interpreted as being essential conditions for the tumor growth (anaplasia of tumor cells) are only secondary changes produced by the abnormal conditions under which the growth is taking place.

The large number of epithelial pearls is a characteristic feature of many carcinomata derived from the skin. In epithelium growing in bloodserum, epithelial pearls are not rarely found. It was possible to determine in a number of cases, as the cause of these formations, the presence of foreign bodies; in this instance, of pieces of bloodserum. At an early period we see well-developed epithelial cells surrounding pieces of bloodserum. Figure 1 shows two such pieces encircled by well-developed, not degenerated epithelial cells. These latter are derived from pigmented epithelium, and therefore carry pigment themselves. This figure represents a relatively early stage, seven days five hours after transplantation of the epithelium into the bloodserum (Loeffler's bloodserum was used). Figure 2 shows a later stage of the same process, twelve days after the operation. The piece of bloodserum in the center is surrounded by several layers of epithelial cells, which are already somewhat advanced in the process of keratinisation. These pearls are not as large as the pearls usually seen in carcinoma, but this difference is probably caused by the different rate of growth in the ordinary and in carcinomatous epithelium. Not all epithelial pearls are caused by the presence of foreign bodies, but the presence of foreign bodies is one of the causes which is liable to produce them. In a former paper I have already pointed out that probably active movements of the epithelium are at work under these conditions. Figure 3 shows a small pearl formed without bloodserum. In its center we find an epithelial cell with a nucleus. The latter is surrounded by protoplasm. The outer part of the cell consists of fibrils arranged in a radiating way. This zone is surrounded by a well-defined membrane. This figure demonstrates one of the changes which may take

place in epithelial cells under these experimental conditions. Similar cells are not infrequently seen in carcinoma. The following figures also are reproduced to show the changes which are taking place in the epithelium under these conditions, and which when occurring in carcinoma have occasionally found a different interpretation. A cell differing from ordinary epithelial cells is represented by Figure 4. It shows an isolated epithelial cell which in the microscopic specimen, however, was surrounded by other epithelial cells. The nucleus shows here a peripheral hyperchromatosis. The chromatin granules, which are all in contact with the nuclear membrane, are connected with each other by threads. These chromatin particles probably were derived from the chromatin of the epithelial cell. As a remote possibility it must be conceded, however, that these chromatin granules were formed by immigrating leucocytes. Such a nucleus is not usually seen in an epithelial cell. This cell was found twelve days after transplantation. In epithelium growing in bloodserum not infrequently cells are seen like the isolated cell in Figure 5. This specimen is from a piece taken from the animal ten days after operation. The space between the isolated cell and the row of epithelial cells near by was probably originally filled by other epithelial cells, which, however, had disappeared. Another fact of interest is that epithelial cells transplanted into bloodserum often take up particles of bloodserum and include them in vacuoles. The nucleus of the cell usually surrounds one side of the vacuole. This fact I have already briefly mentioned in my former paper. The earliest stage in which I found this process to take place is shown in Figures 6 and 7, five and one-half days after transplantation of epithelium into bloodserum. In Figure 6 a large number of cells include pieces of bloodserum. These particles are of different sizes. Most of these cells carry much pigment, as is the rule for pigmented epithelial cells at this period of regeneration.¹

The cells are not yet degenerated, as is especially well seen

¹ L. Loeb, *Über Transplantation von weisser Haut*, etc. *Archiv. f. Entwicklungsmechanik*, Vol. 6, 1897.

in Figure 7, taken from the same piece. Here only a few cells contain bloodserum. Figure 8 shows particles of bloodserum in epithelium ten days after transplantation. The epithelium is quite vacuolar and many epithelial fibers are present in the periphery of the cells. In the vacuolar cells particles of bloodserum of different sizes are situated. It might be suggested that here perhaps a process of intracellular digestion has been taking place, and that the vacuoles were caused by particles of bloodserum which originally were included in these vacuoles. Figure 5 of the same period after transplantation shows bloodserum in a vacuole inside of the isolated cell mentioned before. Figures 9 and 10 show the presence of bloodserum inside the cells at a still later period, twelve days after transplantation. The cells are beginning to form keratinlike fibers. Their center frequently still contains protoplasm and a nucleus; the periphery, however, is often separated from the central part by a clear space. Several cells contain pieces of bloodserum in a sharply defined vacuole. At this stage of regeneration the pigment has disappeared from most cells. By what mechanism does the bloodserum get inside the cells? At early stages we can often see the epithelial cells surrounding particles of bloodserum. In the next stage the bloodserum is inside the cells. Figure 7 (five and one-half days after transplantation) and Figure 11 (twelve days after transplantation) show the epithelium penetrating into the bloodserum.

In Figure 7 no or very few leucocytes were present. In Figure 11 many leucocytes were present, and possibly opened a way for the epithelial cells. It is very probable that both these processes, the penetrating into the bloodserum and the taking up of small parts of it, are produced by slow protoplasmic movements of the epithelial cells. The particles of bloodserum are certainly often so large that leucocytes cannot have carried them into the epithelial cells. Lately Coates¹ and Ricketts² observed in blastomycetic dermatitis, blastomycetes as the center of the epithelial pearls

¹ Coates, W. E. A case of Blastomycetic Dermatitis. *Medicine*, Feb., 1900.

² Ricketts. Oidiomycosis of the Skin. *Journal of Medical Research*, Vol. 6.

(Coates), and blastomycetes inside of epithelial cells (Ricketts). In this case an active growth of blastomycetes into the pearls or cells can, of course, not be absolutely excluded.

A somewhat unusual occurrence is reproduced in Figure 12, where a piece of bloodserum twelve days after transplantation is surrounded by a cell very much resembling the chromatophores found in normal pigmented epithelium.

We have shown that, experimentally, a number of changes can be produced in the regenerating epithelium, which are also found in carcinomatous epithelium. These changes consist in (1) the formation of pearls; (2) certain variations in the structure of epithelial cells; (3) the production of cell inclusions. Two of these changes (Nos. 1 and 3) are directly determined by the presence of foreign bodies. The second change is only indirectly caused by the presence of bloodserum. These experiments may aid us in the interpretation of the structures found in carcinoma. They prove that certain structures are caused by processes which take place in certain epithelial cells alone; that other changes may be produced by the presence of substances foreign to the epithelium. This result suggests the possibility that some of the so-called cell inclusions in carcinoma may be produced in a similar way.

A series of experiments in which pieces of epithelium in bloodserum were removed at different periods of their growth for microscopic examination demonstrated that the changes taking place in the epithelium present a certain regularity with regard to the time at which they are found. Bloodserum is found inside the epithelial cells five days after transplantation. At this period epithelial cells are found at different places in the interstices of the bloodserum. These processes are further advanced at seven days. The cells, however, are still well preserved at this time. From now on more pronounced changes in the epithelial cells begin to take place which are quite apparent after ten days and are very distinct twelve days after the operation. Further experiments will have to be undertaken to determine whether by

certain variations of the methods employed a prolonged growth of the epithelium in bloodserum can be obtained.

Summary.

First: By transplanting epithelium into agar or bloodserum in the living animal changes can be observed resembling changes taking place in carcinomatous epithelium. Of the structural peculiarities thus produced, some are caused by degenerative processes in the epithelial cells, others by inclusion of foreign particles in the cells, still others by foreign bodies determining the arrangement of the epithelial cells (Formation of Epithelial Pearls). These experimental methods will aid us in determining what changes in carcinoma are produced by degeneration of carcinoma cells and what changes may be produced by the presence of foreign bodies.

✓ *Second:* The inclusion of bloodserum in epithelial cells can be observed as early as five days after operation and can still be seen twelve days after operation.

Third: Pigmented epithelial cells transplanted into bloodserum show in the first twelve days the same changes in pigmentation which were found to take place in epithelium when it is regenerated in the usual way, together with connective tissue.

Fourth: The more pronounced degenerative changes in epithelium transplanted into bloodserum begin to take place about the eighth day after transplantation.

Fifth: These experiments prove that the changes found in carcinoma (a part of which may also be found in blastomycetic dermatitis) are not, so far as they have been referred to here, caused by any *specific* influence (for instance, blastomycetes), but are determined by the character of the epithelial cells, under the influence of chemically indifferent foreign substances.

DESCRIPTION OF PLATES.

PLATE IX.

FIG. 1. Epithelium, seven days and five hours after transplantation into bloodserum. a. Pigmented epithelial cells. b. Pigmented epithelial cell including bloodserum. c, c₁, c₂. Bloodserum between epithelial cells. Obj. $\frac{1}{2}$ x Oc. 4.

FIG. 2. Twelve days after transplantation into bloodserum. a. Piece of bloodserum. b, b₁, b₂, b₃, b₄. Epithelial cells around bloodserum. Obj. $\frac{1}{2}$ x Oc. 8.

FIG. 3. From the same piece as Fig. II. a. Striated epithelial cell, surrounded by epithelial cells. b. Obj. $\frac{1}{2}$ x Oc. 8.

FIG. 4. Twelve days after transplantation into bloodserum. Epithelial cell with unusual arrangement of the chromatin. Obj. $\frac{1}{2}$ x Oc. 6.

FIG. 5. Ten days after transplantation into bloodserum. a. Bloodserum. b. Epithelial cell with bloodserum. c. Column of epithelial cells. Obj. $\frac{1}{2}$ x Oc. 4.

PLATE X.

FIG. 6. Epithelium, five and one-half days after transplantation into bloodserum. a. Bloodserum. Most of the epithelial cells include bloodserum. Many of the epithelial cells are pigmented, being derived from black epithelium. Obj. $\frac{1}{2}$ x Oc. 4.

FIG. 7. From the same piece as Fig. VI. a. Bloodserum. Epithelial cells with bloodserum. The majority of the epithelial cells are without bloodserum. Obj. 4mm. x Oc. 4.

PLATE XI.

FIG. 8. Bloodserum in vacuolar epithelial cells, ten days after transplantation. a, aa. Particles of bloodserum. Obj. $\frac{1}{2}$ x Oc. 4.

FIG. 9. From the same piece as Figs. II. and III. Bloodserum in epithelial cells, twelve days after transplantation. a, b, c. Bloodserum in epithelial cells. Obj. $\frac{1}{2}$ x Oc. 4.

FIG. 10. From the same piece. a. Particle of bloodserum in epithelial cell. Obj. $\frac{1}{2}$ x Oc. 4.

FIG. 11. From the same piece. a, a₁. Epithelial cells penetrating into blood serum. b. Bloodserum. c, c₁. Leucocytes. Obj. $\frac{1}{2}$ x Oc. 4.

FIG. 12. From the same piece. a. Cell resembling an epithelial chromatophor surrounding a piece of bloodserum, b. Obj. $\frac{1}{2}$ x Oc. 4.

THE INFLUENCE OF THE SPLEEN ON NATURAL OR ACQUIRED HEMOLYTIC PROPERTIES OF BLOODSERUM.*

ISAAC LEVIN, M.D.

The function of the spleen is not absolutely essential to the preservation of the life of the organism; animals and human beings alike survive its extirpation for an indefinite length of time. Still there are numerous facts, clinical as well as experimental, which indicate that the spleen plays some part in the protection of the body against noxious agents invading it from without. Many infectious diseases (typhoid, relapsing and scarlet fever, pyemia and so on) are accompanied by an acute hyperplasia of the spleen. Ponfick¹ and others showed experimentally that certain particles of pigment injected intravenously are found in great numbers in the spleen. The same is true of bacteria. Wyssokowitsch² found that certain bacteria injected intravenously disappear from the blood within three hours, to be found again in great numbers in the spleen; Metchnikoff³ observed the same phenomenon in *Spirochaete Oberneiri*.

The fact that hyperplasia of the spleen occurs in infectious diseases, and that the spleen seems to amass and consequently arrest the further distribution of microorganisms, led investigators to suppose *a priori*, that the spleen may have a protective influence on the body against infection or intoxication. A number of investigators have approached this question experimentally, but their results do not coincide, for while the results of some have been positive, those of others have been negative.

The method of research pursued by these investigators was the following: They either infected a normal animal and also one whose spleen was extirpated, with the same amount

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An Experimental Study from the Department of Pathology, College of Physicians and Surgeons, Columbia University, N.Y.

of pathogenic bacteria, and compared the results, or else they studied the possibility of artificially immunizing an animal deprived of its spleen against certain kinds of pathogenic bacteria, in the same way as they would a normal animal.

Bardach⁴ succeeded in killing rabbits whose spleens had been extirpated with doses of anthrax bacilli that were not fatal for normal animals. Sudakevitch⁵ obtained similar positive results working on monkeys with spirilla of recurrent fever. Kourloff⁶ infected rabbits deprived of the spleen with different pathogenic bacteria and obtained contradictory results. Tizzoni and Cattani⁷ reported that an animal deprived of its spleen could not be immunized against tetanus bacilli, while Benario⁸ succeeded in immunizing animals without the spleen against different pathogenic bacteria just as well as in normal animals. In a word, there seems to be a great diversity of results in regard to the influence of the spleen on different bacterial infections and on bacterial immunity.

Courmont and Duffau⁹ undertook to investigate the reason for these apparently contradictory results. The plan of their experimentation was the following: They extirpated the spleen of rabbits. Some of the animals were inoculated with different bacteria immediately after the splenectomy, while others were inoculated a certain time after the operation. They found that splenectomized animals, whether the operation were recent or old, succumb more readily than do normal animals to infection with *Bacillus pyocyaneus*. In infection with *Staphylococcus pyogenes*, rabbits with a recent splenectomy succumb more readily than normal rabbits, while an old splenectomy has no effect on them. In infection with streptococcus, on the contrary, rabbits with an old splenectomy succumb more readily. The conclusion which they draw from their work is, that the influence of the spleen differs according to the nature of the poison and the time elapsing between the extirpation of the spleen and the poisoning. The spleen may protect against intoxication, or may not influence it at all, or may even favor it. On the produc-

tion of acquired bacterial immunity, the extirpation of the spleen has, according to their results, no influence.

Similar conclusions have been reached by Blumreich and Jacoby,¹⁰ J. Nicholas and M. Beau.¹¹ The latter experimented on intoxication with mineral and alcaloidal poisons.

As far as the influence of the splenectomy on bacterial immunity is concerned, the results of most experimenters have been uniformly negative. Splenectomized animals are just as easily immunized as normal animals. Tizzoni and Cattani, however, stated that they failed to immunize an animal against tetanus, but these results were also subsequently shown by Benario to be wrong.

The method used by all these authors consisted in immunizing animals normal and splenectomized against certain bacteria, and then ascertaining whether they would withstand the same fatal dose of the bacteria.

Pfeiffer and Marx¹² were the first to use a different method in studying the question of the influence of the spleen on immunity. They immunized a rabbit against cholera bacilli, and then compared the immunizing capacity of the blood serum with the capacity of extracts of different organs of the same animal. They found that during the first few days after immunization, the extract of the spleen had a higher immunizing capacity than the bloodserum. Later the immunizing capacity of the spleen extract became equal to that of the bloodserum. Also, according to their experiments, the extirpation of the spleen has no influence on the development of immunity. Their conclusion is that the spleen is one of the organs contributing to the development of this immunity. In view of the anatomical structure of the spleen, I would suggest that the facts noticed by Pfeiffer and Marx may be due to some kind of a filtering action of the spleen on the blood, which in the beginning of immunization, when the immune bodies are unevenly distributed in the blood, might differ from that at a later stage, when the blood becomes uniform. The results of Pfeiffer and Marx were corroborated by Deutsch¹³ on typhoid bacilli, and Wasserman and Takaki¹⁴ on typhoid bacilli and pneumococci, and Castellani¹⁵ on the dysentery bacilli of Kruse.

Recent investigations on immunity have not only given us a clearer understanding of its mechanism, and showed a difference between immunity against living bacteria on the one hand, and their toxins on the other, but have also proved that the body is able to adapt itself to and subsequently counteract an invasion not only of pathogenic bacteria and their toxins, but also of various kinds of foreign cells or even unorganized matter. The most striking example of such immunity against foreign cells is seen in the acquirement of hemolytic properties by bloodserum. This property of bloodserum to dissolve the hemoglobin from foreign erythrocytes is specific, *i.e.*, a certain serum is lytic only for certain erythrocytes, — and further exists naturally or may be acquired, *i.e.*, if an animal receives a few injections of foreign blood, its serum becomes lytic for the erythrocytes of this blood, even if it were not so normally. Such an animal whose bloodserum has acquired the property of disintegrating foreign erythrocytes may be just as properly considered immune against such erythrocytes as an animal whose bloodserum has acquired the power to kill the cholera bacilli is considered immune against such bacilli.

The relation of the spleen to the formation of red blood cells is undoubtedly very close. Jawein¹⁶ and others have established the fact that the breaking up of erythrocytes is a normal function of the spleen. Thus we seem to be justified in the assumption that the spleen may have some influence upon the natural or acquired hemolytic properties of the bloodserum.

It was in view of these considerations that I undertook to study this question experimentally.

The following is a brief account of my experiments, all of which were performed on rabbits :

In order to make the bloodserum of the animals lytic for erythrocytes of animals of another species, I injected defibrinated bullock's blood into the peritoneal cavity of rabbits. I repeated these injections three to four times at intervals of from three to five days. For the first injection I used six cubic centimeters of defibrinated bullock's blood, for the

second seven cubic centimeters, and for the third and fourth injections eight cubic centimeters of blood.*

To test the hemolytic power of the bloodserum of the rabbits, I added one part of the serum to four parts of bullock's blood diluted one to ten in physiological salt solution.

Bloodserum of the immunized animals in the above dilution induced complete lysis in twenty minutes to two hours, while bloodserum of a normal rabbit is not at all lytic for bullock's erythrocytes.

To test the influence of the spleen on this artificially induced hemolytic power, I performed the following series of experiments:

FIRST SERIES.

Splenectomy on Previously Immunized Animals.

Six rabbits were immunized against bullock's blood, and then the spleen was extirpated; about a week later the serum was tested for its hemolytic power.

Rabbit No. 1 received four intraperitoneal injections of bullock's blood; three days later splenectomy; six days later complete lysis in one hour.

Rabbit No. 2, three intraperitoneal injections; two days later splenectomy; eight days later complete lysis in one hour.

Rabbit No. 3, four injections; two days later splenectomy; five days later lysis in twenty minutes.

Rabbit No. 4, four injections; one day later splenectomy; seven days later lysis in one-half hour.

Rabbit No. 5 three injections; twenty-five days later splenectomy; seven days later lysis in one-half hour.

Rabbit No. 6, three injections; sixty days later splenectomy; five days later lysis in one hour.

The results of these experiments, it will be observed, are uniformly negative, the splenectomy does not have an appreciable effect on a previously acquired hemolytic power of the rabbit's bloodserum.

* Once prepared the rabbits remain immune as long as four months (I did not follow them longer) without any subsequent injection of bullock's blood.

SECOND SERIES.

Immunization after a Recent Splenectomy.

On four rabbits I extirpated the spleen and immediately after it began the immunization.

Rabbit No. 7, splenectomy, three days later three injections at intervals stated above; five days after last injection complete lysis in two hours.

Rabbit No. 8, splenectomy, two days later four injections; seven days after last lysis in two hours.

Rabbit No. 9, splenectomy, one day later three injections; six days after last lysis in one-half hour.

Rabbit No. 10, splenectomy, one day later three injections; seven days after last lysis in one-half hour.

These experiments also uniformly show that a recent splenectomy does not interfere in any way with the acquisition of hemolytic power.

THIRD SERIES.

Immunization a Few Weeks after Splenectomy.

On six rabbits I extirpated the spleen, and a few weeks thereafter the animals were immunized.

Rabbit No. 11, splenectomy, twenty days later four injections at intervals stated above; seven days after last complete lysis in two hours.

Rabbit No. 12, splenectomy, nineteen days later four injections; seven days after last lysis in one-half hour.

Rabbit No. 13, splenectomy, twenty-six days later four injections; eight days after last lysis in one-half hour.

Rabbit No. 14, splenectomy, nineteen days later three injections; six days after last lysis in two hours.

Rabbit No. 15, splenectomy, twenty-four days later three injections; seven days after last lysis in one-half hour.

Rabbit No. 16, splenectomy, thirty-five days later three injections; seven days after last lysis in one-half hour.

The result of this series is identical with that of the second series. Animals with an old splenectomy are just as easily immunized as normal animals.

To ascertain whether there is not at least some quantitative difference in the acquisition of the hemolytic power as

between splenectomized and normal rabbits, I tested the serum of rabbits 9, 10, 15, and 16 after each injection of bullock's blood, and repeated the same for control on normal rabbits. In each case I found the same result — neither after the first nor the second injection is the serum hemolytic, and it always acquires the power after the third injection.

FOURTH SERIES.

Although the experiments described show that the function of the spleen is not indispensable in the acquisition of hemolytic power, it may still be possible that it takes part in it. If this be the case, such action of the spleen ought to become apparent, when the foreign blood is brought in direct contact with the spleen. To accomplish this, I used the following procedure: I opened the abdominal cavity as for a splenectomy, clamped all the blood vessels of the spleen, then with a hypodermic syringe injected one to one and one-half cubic centimeters of bullock's blood, and finally drew out the needle and ligated the spleen *en masse* at the place of the puncture to prevent the bullock's blood from escaping. About twenty minutes later the clamps were opened, the circulation of the spleen was re-established, and the abdominal wound was closed. This operation was repeated on four rabbits. In none of the animals was the serum lytic. I did not repeat the injection in the same spleen because if there is a difference between the immunization through the general circulation, or through a direct contact with the spleen, it must be a striking one. Now if more than one injection into the spleen is necessary in order to immunize an animal, the inference would be plausible that the injection into the spleen acts in the same way as an intravenous injection.

FIFTH SERIES.

Influence of Splenectomy on Natural Hemolysis.

To test the influence of the spleen on natural hemolytic power, I made use of hen's blood. Bloodserum of a normal rabbit is lytic for the erythrocytes of the hen. I tested with

these erythrocytes the bloodserum of rabbits deprived of their spleens. I found that neither a recent nor an old splenectomy impairs the natural hemolytic power of a rabbit's serum against the hen's erythrocytes.

During the progress of my work there appeared a publication by Shibayama,¹⁷ who states that he mixed a ten per cent emulsion of a normal guinea-pig's spleen with defibrinated dog's blood, and found that while guinea-pig's serum is never normally lytic for dog's erythrocytes, the spleen emulsion was lytic for these in some of his animals. I repeated this experiment with a spleen emulsion of a normal rabbit and bullock's erythrocytes, and failed to get a single positive result. This in itself is not necessarily contradictory of Shibayama's results obtained on different animals. But I wish to draw attention to the fact that it is utterly impossible to obtain regularly an emulsion of the spleen without an admixture of erythrocytes of the same animals. Now for these erythrocytes of a guinea-pig, the dog's bloodserum, which is always present in the defibrinated blood, is normally lytic, and this might account for the hemolysis noticed in some of Shibayama's experiments. At any rate, if his conclusion that the spleen is the seat of hemolytic power is correct, it seems strange that his results should not be uniform.

Conclusion.

The conclusions that may be drawn from this research are as follows: The spleen is undoubtedly not indispensable for the acquired or the natural hemolytic properties of the bloodserum. Nor, so far as these experiments go, does it appear to elaborate these substances upon which either the normal or artificial hemolytic properties of the bloodserum depend. This last question, however, needs further research with a different technique.

(I wish in closing to express my gratitude to Dr. T. Mitchell Prudden, in whose laboratory this research was carried out.)

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SPINDLE-SHAPED DILATATIONS AND TORTUOSITY OF THE URETERS IN THE FETUS.¹

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Certain peculiarities in the fetal ureters which I had noticed from time to time and had not seen referred to in the commonly used text-books, led me to make a number of dissections to determine the frequency of these peculiarities.

Among thirteen fetuses whose age was from seven to nine months, the ureters presented *more or less distinct spindle-shaped dilatations in all* but two (2). The spindle is usually larger on the right than on the left side, in two (2) it is only present on the left side, and in three (3) only on the right.

There was no difference in the sexes as far as could be learned.

Where the ureter crosses the iliac vessels and is bent upon itself there is a slight narrowing, and the enlargement extends for from one and one-half to three centimeters. The dilated portion is from one and one-half to two and one-half times as great in width as the rest of the ureter, and is flattened out.

In three of these fetuses the ureter has serpentine curves, or is tortuous. In my experience curves and tortuosities of the ureters are more frequent in younger fetuses.

In none of the fetuses examined were there any evidences of atresia in the urinary tract.

Among twenty-two fetuses, varying in age from three to seven months, there are spindle-shaped dilatations or tortuosities or both, in all but four (4). The spindles are nearly always just above the point where the ureter crosses the pelvic brim.

The curves are sometimes long and serpentine, at other

¹ Read, March 28, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, Ohio.

times the ureters are quite tortuous and spirally curved; occasionally there are from two to four corkscrew turns near the kidney.

In but four cases does the ureter follow a perfectly straight course from the kidney to the pelvic brim without dilatations.

It would seem from the above statements that it is justifiable to conclude that spindle-shaped dilatations and tortuosities of the ureter are nearly constant in the fetus, and are therefore normal.

If we may be permitted to speculate as to their causation, we might say that the dilatation just above the brim of the pelvis is due to obstruction to the escape of urine from the slight narrowing, and from the curve of the ureter as it crosses the iliac vessels. This explanation would naturally occur to one.

As regards the curves and tortuosities, it may be assumed that they disappear with the growth in length of the body, for they do not exist in adults.

Luschka was probably the first to refer to spindle-shaped dilatations of the ureter in adults. He states (*Anat. des Bauches*, 1863, p. 204) that they occur in all cases.

Rauber and Quain state that the ureter is frequently enlarged near its lower end.

More recently Schwalbe (*Verhandl. d. Anatom. Gesellsch. zu Berlin*, 1896. Jena, 1896) describes as normal a fusiform dilatation of the ureter just above the pelvic brim and gives as a reason for its existence the angulation which is present here, which interferes with the flow of urine.

The ureter, according to this writer, has a *pars abdominalis* and a *pars pelvina*.

In quadrupeds in which there is no angulation (*flexura marginalis*, Schwalbe) and no *pars pelvina*, there are no dilatations. Schwalbe ascribes the angulation to the erect posture of man.

He found the spindles also in two fetuses and three newborn children, and decides that these peculiarities are inherited, and are not the result of individual development.

Solger refers to these dilatations (Klin. Handbuch. d. Harn. und Sexual Organe, Leipzig, 1894, Bd. I.).

He also describes them in six fetuses and alludes to the tortuosities of the ureter (Anatom. Anzeiger., Bd. XII, p. 347).

It seems possible that these tortuosities might, in some cases, favor the development of hydronephrosis.

ON THE OCCURRENCE OF CARCINOMA AND TUBERCULOSIS
IN THE SAME ORGAN OR TISSUE.¹

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Though many instances of the occurrence of both carcinoma and tuberculosis in the same individual have been reported, the occurrence of the two diseases in the same organ or tissue is relatively rare, especially in certain situations. It seems worth while, therefore, to put on record five personally observed cases, and at the same time to call attention to certain aspects of the association of the two diseases which have been but little dwelt upon.

Following is a description of the cases observed:

Case I.—Recurrent carcinoma with apparently primary tuberculosis in the mammary gland; metastases of both diseases to the axillary lymph glands of the same side.

The specimen consists of a mass of fat, on one surface of which is a skin area, on the other the greater portion of the major pectoral muscle. The skin area measures twelve by six centimeters. It contains the nipple, which is approximately normal in appearance. At one point it contains a depressed cicatrix five centimeters in length. Beneath the cicatrix can be felt a dense new growth reaching to the skin. On section the growth is small, measuring about two centimeters in diameter, and irregularly scattered through the fat. It is of a grayish-white color and contains practically no necroses. In the fat adjoining the breast, which apparently represents the axillary fat, there are a number of enlarged hard glands, varying from one to one and one-half centimeters in diameter, and completely infiltrated with new growth. The pectoral muscle is apparently unaffected.

Anatomical Diagnosis.—Recurrent carcinoma of the breast with metastases to the axillary glands.

¹ Read, March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, Ohio.

Microscopic examination of the breast tumor shows that in a few places it is largely composed of fibro-connective tissue, but for the most part the connective tissue forms a dense fasciculated stroma enclosing rather long but very irregular columns of atypical epithelial cells, in places cuboidal in shape, in other places, especially where there is a single column of these cells lying in a dense connective tissue fenestra, they are elongated. These cells all stain rather deeply in the hematoxylin. Practically all the nuclei are granular in appearance, but some show a nucleolus. Sections from six different parts of the tumor were carefully examined, all of which revealed the same typical picture of scirrhus carcinoma. In part of one section the alveoli are more circular. Here the cells are quite small and more nearly circular. Here also there is a considerable accumulation of small dark staining lymphoid cells in the stroma. In addition there were found six typical miliary tubercles at the periphery of the carcinomatous process. The centers of these tubercles are taken up with small accumulations of rather pale epithelioid cells, and from two to six typical giant cells, mostly with peripherally arranged nuclei. Surrounding these epithelioid and giant cells is a fair zone of lymphoid cells, but this zone is sparse on the side where the epithelial invasion comes very close to the tubercles. At one place there is a miliary tubercle surrounded on all sides by the carcinoma; it also consists of the central accumulation of pale epithelioid cells with three typical giant cells, and surrounded almost entirely by a fair zone of small dark staining lymphoid cells. On one side, however, it is protected by a connective tissue stroma as yet uninvaded by the carcinoma. Careful search was made through many paraffin sections cut in series and an occasional tubercle bacillus was demonstrated in these tubercles.

The microscopic examination of an axillary gland shows that practically the whole gland is infiltrated with a new growth quite cellular in character. The cells which are epithelial occur mostly in long narrow alveoli, and the connective tissue stroma between the alveoli is rather moderate in

amount. The tumor cells are mostly of the round type; they are irregular in a few places, however, where there has been mutual compression, and where the stroma is more dense. In the outer zone of the section at one point is an elliptical miliary tubercle, one millimeter long by one-third millimeter wide, practically surrounded by the carcinomatous process. The tubercle is made up of an outer zone of small round lymphoid cells, the center is occupied with a large number of epithelioid cells, and in the midst of these are five distinct giant cells. This tubercle being accidentally found, the remainder of the gland was sectioned and several tubercles, similar to the one described, were found. Some of these were not surrounded at all, others partly, and four entirely by the new growth. All of these tubercles are near or on the periphery of the neoplasm. Here also paraffin sections were cut and a few tubercle bacilli found in sections stained by carbolfuchsin.

Microscopic Diagnosis. — Scirrhus carcinoma of the breast, associated with a tuberculous infection and the two diseases metastatic to the neighboring lymph glands.

The only cases which are strictly comparable to this are the two cases reported by Warthin. Our case differs from his in that it occurred in a recurrent carcinoma and not in the original growth, and also in the fact that metastases of both carcinoma and tuberculosis occurred in the axillary glands on the same side. In Warthin's cases the combination occurred in the mammary gland alone.

After a careful search of the literature Warthin was unable to find a "single undoubted instance" of the combination of carcinoma and primary tuberculosis of the breast. His cases may be considered unique in that respect, and as far as we could determine our case is the third of this character. Pilliet and Peatot have, however, reported a case of epithelioma of the breast with a fistulous opening of a tuberculous nature. In our case the carcinoma was evidently the primary process, and the tuberculosis due to secondary invasion. The tubercle bacilli may have been introduced at the operation for the removal of the primary tumor and may have

remained latent in the wound. This would seem unlikely, however, and it is much more probable that they were carried by the blood or lymph from some other part of the body. In Warthin's cases the possibility of their entrance through the milk ducts could not be excluded; nor could it in our case, as the nipple was not removed until the second operation, and the first one may have damaged the main ducts but little if at all.

Case II.—Carcinoma and tuberculosis in an axillary lymph gland. An accidental find during routine microscopic examination.

Anatomical Diagnosis.—Scirrhus carcinoma of the breast with metastases to the axillary lymph glands.

Microscopic description of the axillary gland. The gland shows but little normal tissue remaining, the greater part of the gland being replaced by a new growth made up of atypical epithelial cells arranged in alveoli, which as a rule are long and narrow. These alveoli are separated from one another by a moderate amount of connective tissue stroma which has caused but little distortion in the shape of the cancer cells. In one end of the gland there are noticed about twenty very early miliary tubercles. These are entirely within gland tissue which is not yet involved by the carcinoma. There is but little normal gland tissue between the carcinomatous and tuberculous processes, however. Three or four of these tubercles show commencing degenerative changes in their centers. They are made up for the most part of epithelioid cells, among which are noticed three or four giant cells. They are surrounded on the outside by the small round cells of the gland tissue. The tissue showing these tubercles could not be recovered, therefore no study for tubercle bacilli could be undertaken.

Microscopic Diagnosis.—Metastatic carcinoma of an axillary lymph gland associated with tuberculosis.

This appears to be an instance of the occurrence of the two diseases in the same tissue, merely as a coincidence, and independently of one another. It seems unlikely that the tubercle bacilli were transmitted from the mammary gland,

as there were no signs of a tuberculous lesion in the primary growth after most careful search. It is probable that tubercle bacilli were carried to the gland either by the lymph or blood current independently from the carcinoma cells, and that the two diseases developed independently of one another.

Case III.—Carcinoma in the mammary gland with the combination of carcinoma and tuberculosis in a lymph gland, probably from the axilla.

Anatomical Diagnosis.—Primary scirrhus carcinoma of left mammary gland. Metastases to the axillary, bronchial, retroperitoneal, and perigastric lymph glands. Extensive metastases to both pleuræ. Post-mortem change in the heart muscle with fragmentation of the myocardium. Extensive double hydrothorax with atelectasis and edema of the lungs. Cloudy swelling of the liver with slight chronic passive congestion. Slight chronic interstitial nephritis. Multiple myomata of the uterus.

In the routine examination of the tissues from this autopsy there was accidentally found the lymph gland to be described. What part of the body it came from cannot definitely be known, but it is safe to say that it is not a bronchial gland, for sections from four different parts of the gland showed no deposit of carbon pigment.

Microscopic examination of sections from different parts of the breast tumor showed it to be a typical scirrhus carcinoma, but revealed no evidences of tuberculosis.

Microscopic examination of the gland in question showed a narrow zone of fairly normal gland tissue about part of the periphery of the section. The central portion of the gland is taken up mostly by six or seven caseous areas of different sizes. Each of these caseous areas seems well walled off by a connective tissue zone. There are no evidences of active tubercle formation, but on the contrary the process seems old and entirely latent, judging from the well marked zone of connective tissue surrounding each caseous focus. Careful search was made through several of these old foci, in paraffin sections stained with carbolfuchsin, and in one of them five or six typical tubercle bacilli were found. Invading the

gland tissue between these different tuberculous areas and the connective tissue about them, and even growing through into the caseous homogeneous centers, are seen epithelial cells arranged in the alveolar manner characteristic of carcinoma. The connective tissue stroma between these columns of cells makes them in places very distinct.

Microscopic Diagnosis.—Quiescent tuberculosis of a lymph gland secondarily invaded by metastatic carcinoma.

This case merely illustrates that a quiescent tuberculous area may be invaded by carcinoma just like other tissues.

Crawford has reported a case closely allied to these last two cases of scirrhus carcinoma of the breast with an abscess, probably tuberculous, though this was not proven, and both metastatic carcinoma and tuberculosis in the axillary lymph glands of the same side. Both giant cells and tubercle bacilli were found in the lymph gland.

Case IV.—Adeno-carcinoma with tuberculosis in the sigmoid flexure, and the same combination in the liver and kidney.

Anatomical Diagnosis.—Primary adeno-carcinoma of the sigmoid flexure with metastases in the liver and the left kidney. Perforation into the bladder. Brown atrophy of the heart muscle. Emphysema of both lungs with chronic tuberculosis, especially in the left. Acute vegetative endocarditis of the aortic valve. Acute spleen tumor. Multiple infarcts of the spleen and kidneys.

The gross appearances of the new growth, liver, and left kidney taken from the autopsy protocol are as follows:

Commencing about twelve centimeters above the anus, the wall of the sigmoid flexure is occupied by an ulcerative new growth which covers a triangular area fifteen by fifteen by ten centimeters in extent. The floor of this is partly ulcerated and covered with a greenish necrotic mass, and is partly occupied by yellowish polypoid masses of new growth. The fat immediately about the intestine at this point shows infiltration with new growth. At one point the floor has ulcerated entirely away so that the intestine communicates with the bladder.

The liver is free from adhesions. The surface is somewhat irregular. In several places there can be seen projecting from the surface yellowish nodules varying from one to two centimeters in diameter, and some of them distinctly umbilicated. On section these nodules are seen to extend into the liver substance for a depth almost corresponding to their surface diameter. They are composed of a peripheral yellowish-white zone, and a central reddish-yellow necrotic zone. The liver substance between them is a trifle granular looking. The central vessels of the lobules are dilated.

Left Kidney.—Fatty capsule large in amount. Fibrous capsule strips off easily, leaving a smooth mottled surface. The fetal lobulations are well marked. The surface of the organ is pale in places, yellow in other places, and distinctly hemorrhagic. In one or two places projecting nodules are seen which on section are wedge-shaped, having a white central portion and a congested outer zone. At one point in the cortex is a yellowish-white tumor nodule three millimeters in diameter. On section of this kidney the cortex is swollen and of a yellowish-red color. The glomeruli are visible, and the medullary portion is somewhat compressed.

Microscopic examination of the new growth in the sigmoid flexure shows it to be an alveolar one made up of large irregular alveoli, separated from one another by a small amount of connective tissue. These alveoli are irregular and are lined, but not filled, by columnar epithelial cells. They form gland-like spaces which in places contain epithelial debris. In places the tumor is infiltrated with polynuclear leucocytes. The new growth projects into the lumen of the intestine and is somewhat fungoid in character. There is also a tuberculous process present in the new growth, which is working its way between the intestinal wall and the fungoid edge of the new growth, undermining the latter. There are two or three fairly definite tubercles in the new growth with broken-down centers, a zone of epithelioid cells with one or two indefinite giant cells, and an irregular outer zone of small lymphoid cells. For the most

part this process is made up of broken-down pink staining caseous material with cell remnants.

Paraffin sections stained with carbolfuchsin and Gabbett's methylene blue were studied and a few very definite tubercle bacilli were found in one of these caseous areas.

Microscopic examination of the liver shows a slight but perceptible increase in connective tissue, the portal spaces being infiltrated with small round cells and polynuclear leucocytes. The central veins of the liver and surrounding capillaries are in places dilated with a corresponding compression of the liver cells. The liver cells are swollen and granular. In places there are distinct areas of focal necrosis, surrounding some of which large phagocytic cells can be made out. Some of the liver cells show a moderate degree of fatty change. A good many of the small bile ducts appear to contain polynuclear leucocytes, and it seems likely that the inflammatory changes in the portal systems may be secondary to a cholangitis. The metastatic carcinoma in the liver has, in the main, a similar appearance to the primary growth in the sigmoid flexure. In one field there are noticed two very definite miliary tubercles. They are entirely surrounded by liver substance, but lie very close to the periphery of the neoformation. The centres of these tubercles contain some caseous debris; around this are large pale epithelioid cells with three distinct giant cells having peripherally arranged nuclei, and then an outer zone of small dark staining lymphoid cells. The lymphoid zones of the two tubercles join each other. Besides these two definite tubercles there are in different parts of the new growth collections of an almost homogenous pink-staining material containing some remains of broken down cells. These necrotic foci occupy, in nearly every instance, the interiors of the cancer alveoli, which, as before stated, are not filled but simply lined with columnar epithelial cells. In one or two places a caseous mass is in contact at one point with the stroma where a few epithelioid cells and polynuclears are to be made out. Paraffin sections were stained and studied for tubercle bacilli, and a few were found in these caseous masses.

Microscopic examination of the kidney shows a slight increase in connective tissue. There are areas in the organ in which groups of tubules have connective tissue between them. They are infiltrated with polynuclear leucocytes. In some of these tubules bacterial plugs can be seen. The kidney cells throughout the organ are greatly swollen and granular. There is an occasional fibroid glomerulus. Some of the larger blood vessels show distinct thickening of their inner coats. The areas seen with the naked eye as infarctions are made out microscopically as completely necrotic zones containing a few polynuclear leucocytes. The metastatic new growth is similar to the original in the intestine. Between this new growth and the kidney substance on one side a tuberculous process is noted, first some broken down cells, then epithelioid cells, and next lymphoid cells. No giant cells seen here. In another field three very early miliary tubercles were seen, one of which contains a giant cell. As no other piece of kidney tissue having tuberculosis could be found in the autopsy material, no attempt was made to demonstrate tubercle bacilli in the kidney.

Microscopic Diagnosis. — Primary adeno-carcinoma of the sigmoid flexure secondarily infected with tuberculosis, and both diseases metastatic to the liver and left kidney.

This case is probably similar to a series of reported cases in which carcinomata of some part of the alimentary tract became inoculated with tuberculosis. Baumgarten, Lubarsch, Friedländer, and Dalton have each described such cases in the intestine. Nægeli also has reported three cases of the combination in the intestinal tract. He argues that the tuberculosis was primary in each instance. Claude and Friedländer have each reported an interesting case of the hybrid in the stomach. Zenker and Cordua have reported similar cases in the esophagus. In all of these cases, with the exception of Nægeli's, it seems likely that the mechanism was the same, that the tuberculous process was the secondary one and was due to swallowing infected sputum, by means of which the new growth became inoculated. The possibility of transmission by the blood current must be admitted,

but seems unlikely. In our case the cancerous area was the only portion of the intestine which was tuberculous, and the case differed from most reported cases in the fact that double metastases occurred both to the liver and kidney. The intimate relation between the two diseases in these latter organs makes it almost certain that both diseases were transmitted in the same way and at the same time. It is also to be noted that as far as the tuberculous lesions in the liver were concerned these did not present a typical tuberculous appearance, and but for the presence of tubercle bacilli their nature might not have been suspected.

Case V. — Adeno-carcinoma of the prostate with tuberculosis and carcinoma in the lungs, liver, spleen, adrenals, bronchial lymph glands, and retroperitoneal hemolymph gland.

Revised Anatomical Diagnosis. — Primary adeno-carcinoma of the prostate with multiple metastases to the ribs, spinal column, ilia, cranial bones, both adrenals, retroperitoneal lymph glands, liver, spleen, back muscles, and lungs. Chronic cystitis. Ascending urinary infection (ureteritis, pyelitis, and nephritis). Double acute pleurisy with effusion. Tuberculosis of the apices of both lungs with fresh tuberculous pneumonia. Tuberculosis of the bronchial glands and kidneys. Concurrence of tuberculosis and carcinoma in the lesions in the lung, liver, spleen, adrenals, bronchial glands, and a retroperitoneal hemolymph gland. Cloudy swelling of the liver. General arterio-sclerosis. Chronic passive congestion of all of the organs.

The gross appearances of the different organs in which both of the diseases occur, are taken from the autopsy protocol, and are as follows:

Left Lung. — Adherent at its extreme apex, and also at the base by old firm adhesions. At the extreme apex there is a calcareous nodule surrounded by fibrous tissue, the whole area being about one centimeter in diameter. There are a few pleural tubercles, many felt better than seen. In the upper lobe near the hilum is a distinct area of consolidation, which has a grayish somewhat translucent appearance, and

contains definite conglomerate tubercles. It shows diffuse caseation in places. The rest of the upper lobe is pigmented and slightly congested. The lower lobe is pigmented and partly congested, but free from consolidation. It is almost airless, however. The bronchi contain sticky mucus. Their mucous membrane is congested. The larger blood-vessels are free from clot.

Right Lung.—The surface is covered by a partly organized fibrous exudate, particularly posteriorly. At the extreme apex there is an area of calcification as in the other lung. The upper lobe is free from consolidation, except at one point, where on section is a pea-sized caseous area. Aside from this the upper and middle lobes are almost airless from compression by the pleural exudate. Lower lobe completely atelectatic. Bronchi and larger blood vessels as in other lung. The bronchial glands are enlarged, pigmented, and some showed diffuse caseous areas.

Microscopic Description. Lung.—The pleural connective tissue shows marked dilatation of its blood vessels, and is infiltrated with small round cells, red blood corpuscles, and polynuclear leucocytes. In places it contains definite tubercle nodules, some very early miliary tubercles, and others older and caseous. The surface of the pleura is covered with an exudate composed of degenerated fibrin, in the meshes of which are polynuclear leucocytes, red blood corpuscles, and small round cells. There are also many epithelioid cells in this fibrinous exudate. The appearances of the lung differ in different parts: in some places the appearance is normal, in others the air spaces are very small, and yet in other places the lung is completely atelectatic and the seat of tuberculosis and new growth. The larger tubercles are caseous and all have the typical structure, being made up of epithelioid and lymphoid cells and containing giant cells. A moderate amount of carbon pigment is noticed in different places in the lung. There is noticed about many of the blood vessels an alveolar new growth made up of atypical cylindrical cells having a gland-like arrangement. The cells lining those alveoli are from one to three in depth. The new

growth follows the distribution of the perivascular lymph spaces, and probably was brought to the lung by the lymphatic system. In places miliary tubercles lie adjoining the new growth, at times in actual contact with it. Paraffin sections stained by Novy's method showed many tubercle bacilli in the tubercular areas.

The liver is free from adhesions. The surface is smooth and of a brownish color. On the surface a number of grayish-white circular nodules are seen; these vary from one to two millimeters in diameter. On section they have a homogeneous grayish color. Those nodules have neither the appearance of tubercles nor gummata, and are quite firm in consistency. They look like tumor nodules. The liver on section is brownish in color, the lobules indistinct, the centers of the lobules slightly congested.

Microscopic Description. — The central veins and the capillaries about them are filled with blood, and the bordering liver cells show the consequent effects of pressure. There is some deposition of blood pigment in some of the cells. In different places in the liver substance typical miliary tubercles are noticed in which tubercle bacilli were demonstrated. At one place there is an almost circular nodule of new growth, similar in character to that described in the lung. Here no definite relation could be made out between the distribution of the new growth and that of the blood or lymph vessels. Some tubercles lie just between the new growth and the liver substance.

Spleen. — The spleen is free from adhesions. The capsule is irregularly thickened. There is an occasional nodule on its surface having the appearance of a tubercle. The consistency of the organ is very firm. On section the pulp is not increased. There is a marked increase in the trabecular substance. There are grayish, somewhat translucent nodules scattered through the organ, possibly tubercles, but they may be enlarged Malphigian bodies, or tumor metastases.

Microscopic Description. There is considerable thickening of the trabeculæ. In places this is associated with a deposit of blood pigment. For the most part, however, the thicken-

ing of the trabeculæ is intimately associated with new growth, and might really be said to be due to the proliferation of the connective tissue for the purpose of forming a stroma for the new growth. The growth in the spleen is much more scirrhous than in the other organs with the exception of the prostate. In some places the metastases have a very distinct perivascular distribution which argues strongly for lymphatic transportation. In one or two places, however, patches of new growth with the general distribution and extent of the Malpighian bodies are observed. A few miliary tubercles with caseous centers are noticed, in which typical tubercle bacilli were found; the tumor cells are intermingled with the outer zone of small round lymphoid cells of the tubercles. Practically no normal spleen tissue remains.

Adrenals. — The adrenals are very slightly enlarged. On section they are infiltrated with a grayish-white nodular new growth, which is homogeneous in appearance. There are no signs of caseation. Neither adrenal is entirely destroyed by the new growth.

Microscopic Description. — The blood vessels are thickened and distended with blood, otherwise the uninvolved gland substance seems normal. There are several areas of new growth which have replaced the gland substance. The new growth is similar to that already described in the other organs. It has a very distinct perivascular arrangement, and the most extensive nodules are situated in the region of the large vessels. In one or two places the tumor nodules can be seen in distended lymph spaces. Two miliary tubercles with breaking down centers were noticed, around which are epithelioid and lymphoid cells. Tubercle bacilli were found. These tubercles are situated in the normal gland tissue and not very near the new growth.

Retroperitoneal Lymph Glands. — These glands are matted together into a single large mass, which is infiltrated with a grayish growth similar to that seen in the other organs. The mass measures nine by four by two centimeters.

Microscopic Description. — The combination of carcinoma and tuberculosis was found in a hemolymph gland from this

group. In the affected gland the blood spaces and the small vessels are all distended with blood. There is present in the gland a new growth similar in all respects to that described in the other organs. Here again it seems to have the same perivascular origin. Aside from its distribution about the blood vessels it is also found forming a definite capsule of new growth about the greater portion of the gland, this appearance being apparently due to an infiltration of the gland capsule with the new growth. Three or four typical miliary tubercles containing bacilli were noticed in the gland. These tubercles are associated with the nodes of lymphoid tissue and are not very near the new growth.

Bronchial Glands. — These glands are enlarged, pigmented, and show some diffuse caseous areas. Microscopically one gland is practically occupied by the new growth, which is the same as already described in other organs, and the same perivascular arrangement can be made out. Considerable carbon pigment is noted in the connective tissue framework in different places. In one end of the section there is a small area of gland tissue, but this is mostly taken up by caseous miliary tubercles in which tubercle bacilli were demonstrated. Another bronchial gland shows complete tuberculous caseation, but no new growth.

Microscopic Diagnosis. — Adeno-carcinoma and tuberculosis of the lungs, bronchial glands, retroperitoneal hemolymph glands, adrenal, liver, and spleen, secondary to primary carcinoma of the prostate, and primary tuberculosis of the lungs.

We were unable to find another case recorded in the literature where these two diseases were associated in so many different organs. In fact in nearly every case that we have found reported, the combination existed only in one organ or tissue. In this last case the tuberculosis is undoubtedly primary in the apices of the lungs. The adeno-carcinoma was without doubt primary in the prostate. The facts pointing to this being that it is much more extensive and scirrhus in character in that organ than in any of the other locations, and that extensive bone metastases are well known in con-

nection with certain malignant new growths of the prostate. The prostate being the primary seat of the carcinoma, the metastases probably first passed to the retroperitoneal lymph glands, then to the adrenals, spleen, liver, bronchial glands, lungs, and bones. The tuberculous infection probably passed downward from the lung apices and involved the organs as already described. This was doubtless a blood infection, as usual. No sign of tuberculosis could be discovered in the prostate. In the other organs where the combination exists the lesions are noted in some places intimately intermingled, in other places lying side by side, and yet in other places they occupied different seats in the organ. Which had been the primary lesion in the different organs could not be determined and is probably of little consequence. The possibility of this tumor and its metastases being endothelioma was considered, but as we could not definitely establish endothelial proliferation in the peripheral portions of the growth we hold to the opinion of an adeno-carcinoma.

Aside from the localities affected in the cases of carcinoma and tuberculosis mentioned, the combination of the two diseases in other parts of the body has been fairly frequently described.

Garres, Crone, Baumgarten, and Zenker have reported cases of carcinoma of the larynx complicated with tuberculosis. The combination in lung, caverns in chronic tuberculous subjects has been reported by Lubarsch, Menetrier, Wolf, Schwalbe, and Friedländer. Franqué has reported it in the uterus and Frerichs in the liver. Hang cites a case of both diseases on the lobe of the ear.

The combination of the two diseases in the skin seems to be the most common form. Steinhauser has collected from the literature eighty-three cases of lupus and carcinoma of the skin and reported five new cases. Raymond, Bidault, Vidal and Leloir, and Desbonnets have also given careful study to this condition in the skin. Ribbert reports eleven cases of the hybrid in different parts of the body, but from the descriptions Clements thinks that only one is a true case of the combination, as the diagnosis was made in most of the

cases upon the finding giant cells alone and no other origin for these than from tuberculosis was considered.

Cases of sarcoma complicated by tuberculosis have been carefully reported by Ricker, Trendweiler, and Iscovesco. Cone and Reich have each reported a case of glioma complicated with tuberculosis, and Clements has reported a case of endothelioma of the parotid gland associated with tuberculosis. In nearly all of the cases here cited tubercle bacilli were demonstrated.

Two main questions of interest arise from a study of these cases:

1. The mode of origin of the hybrid disease.
2. The question as to whether there is any real antagonism between carcinoma and tuberculosis.

It seems quite certain from a study of our cases and of the literature that either the carcinoma or the tuberculosis may appear first in the affected tissue, or that both may appear at the same time. Lubarsch, who has probably done the most work in this subject, has mentioned five possible combinations of the two diseases.

1. Simple coincidence. The diseases having no apparent action the one upon the other.
2. Metastatic carcinoma developing secondarily upon a recent or old tuberculous focus.
3. A tubercular infection becoming engrafted on a cancer in full evolution.
4. Chronic progressive tuberculosis on which develops a cancer.
5. The simultaneous development of both cancer and tuberculosis.

It is probable that in some regions one form of the combination is especially apt to occur, and in other regions another, the difference depending partly on the anatomical peculiarities and partly on the susceptibility of the tissue to one or the other of the diseases.

In most of the cases of lupus and carcinoma that have been reported, the writers believe that the lupus was the

primary condition and acted as an exciting cause for epithelial proliferation. Cancer developing upon completely or partly cicatrized lupus Steinhäuser calls "lupus scar carcinoma," and another form which he calls "lupus carcinoma" is where cancer develops on lupus in full activity, either at the edge or amid the fresh lupoid granulations. Desbonnets has carefully studied cancer developing in healed lupus, and Raymond has noted the preference of cancer to develop on fresh lupus. These two observers with many others have shown that with lupus there are often epithelial growths of a benign nature, sometimes papillomatous, and later these may develop into true carcinoma.

Principally from the study of the hybrid in the skin and mucous membrane Ribbert founded his well-known theory of loss of cell restraint as the cause of cancer. He thinks tuberculosis always the primary condition. In those cases where the tubercles are very few and small he thinks carcinoma was much more rapid in growth than the tuberculosis. It seems to us that in this he is fitting the facts to his theory rather than impartially commenting on them.

The authors who reported the cases of the hybrid in the lung concede that the tuberculous condition without doubt existed first, but do not all consider it as the cause of cancer developing later. Cases of cancer developing upon a primary tuberculous focus in other situations are rare. Case III. of this paper is added as another instance of this kind. Cases of a definite secondary tuberculous infection of a carcinoma are those mentioned by Claude, Friedländer, Baumgarten, Crone, and Zenker, and Cases I. and IV. of our series. It is to be noted that many of these cases occurred in the alimentary tract where excellent opportunity for infection by swallowed sputum was present. The explanation that is accepted by most writers, for these cases, is that the cancer becomes a point of lessened resistance favoring the development of the tubercle bacillus, which comes either from the outside world or from some other part of the body.

Regarding the evidence of antagonism between carcinoma and tuberculosis numerous statements are found in the

literature, and some figures bearing on the subject have been published.

Baumes, C. Paul, G. de Mussy, Laveran, and Damaschino have mentioned facts which tended to show that there was a reciprocal resistance between the two diseases. Williams has said "that it is very rare to find both diseases in active process in the same individual" and "it is obvious that there is a certain antagonism between active tuberculosis and cancer." Percy Kidd said, "It is uncommon to find evidences of simultaneous activity." Most authors of to-day do not seem to consider that an antagonism between the two diseases exists.

From Lubarsch's figures, based upon six thousand five hundred and thirty-six autopsies, we find that carcinoma occurred one hundred and seventeen times in two thousand six hundred and sixty-eight cases of active tuberculosis, or in four and four-tenths per cent. Zahn has recently reviewed six thousand three hundred and twenty autopsy records, and in one thousand eight hundred and ninety-three cases of active tuberculosis he found that seventy-six, or four and one one-hundredths per cent, also had carcinoma. Loeb in six hundred and six cases of either cancer or tuberculosis found the two diseases in the same person in less than one-fifth of the cases. Dürck in five hundred and fifty-nine cases of carcinoma found evidences of tuberculosis in one hundred and seventeen. These proportions are also confirmed by Cordua and Zenker. In our own records of four hundred and fifty autopsies we found ninety-seven cases of active tuberculosis, six or six and two-tenths per cent of which also had carcinoma — a somewhat higher proportion than Lubarsch or Zahn reported, but we examined less than seven per cent of the number than either of these men considered.

It seems to us that in considering the supposed antagonism, a number of important factors must be taken into consideration. The most important of these are:

1. That cases of active tuberculosis occur for the most part at a time of life before carcinoma becomes prevalent.

Though we could find no statistics covering a sufficiently

large number of cases of death from tuberculosis in general, yet we found very good figures showing the death rate at different ages from phthisis, namely, Barié's figures based upon ten thousand six hundred and forty-nine deaths from pulmonary tuberculosis. From these figures we find that eighty-two and two-tenths per cent of cases of phthisis are fatal before the age of fifty. Other writers, especially Percy Kidd and Strauss, have given figures yet higher than these. On the other hand, Reiche has given us a table based upon eleven thousand nine hundred and thirty deaths from cancer, showing the mortality at different ages, and from this table we find that seventy-three and forty-four one-hundredths per cent of deaths from cancer occur after the age of fifty. Williams and Laspeyres have given figures higher than these. These two instances are cited as being rather conservative, and plainly show that tuberculosis is distinctly an active disease before fifty, and carcinoma after fifty. In other words, between eighty and ninety per cent of deaths from tuberculosis occur before fifty, and seventy-five to ninety per cent of deaths from cancer occur after fifty. We also learn from Reiche's and Laspeyres's figures that in a large percentage of the cases of cancer before the age of fifty, the cancer occurred in the uterus, where tuberculosis is of relatively infrequent occurrence.

2. That the figures showing that carcinoma and tuberculosis apparently exclude one another are based altogether on cases of active tuberculosis, and do not take into account latent or healed tuberculosis. If these latter forms are taken into account it is evident that the two diseases must be very frequently associated in the same individual, as Nægeli's figures showed that ninety-two and nine-tenths per cent of all adults over eighteen have either active, latent, or healed tuberculosis.

3. That the organs most frequently affected by tuberculosis are not the organs most frequently attacked by carcinoma.

Williams has given the distribution in the body of seven thousand two hundred and ninety-seven cases of primary

cancer, and in over eighty per cent of the cases it was found in locations where tuberculosis is of relatively infrequent occurrence, namely, in the breast, uterus and prostate, tongue, mouth and lower lip, esophagus, stomach, external genitalia, bladder, and superior maxilla. The other locations which he mentioned are the skin, liver, intestines, rectum and anus, testes and ovary, larynx, and a few odd places. These we have excluded, as they are seats where tuberculosis is fairly common.

On the other hand, tuberculosis in adult life is nearly always primary in the lung where but few cases of carcinoma occur. We would conclude, therefore, that there is no real antagonism between carcinoma and tuberculosis.

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NEW AND SIMPLE MEDIA FOR THE DIFFERENTIATION OF
THE COLONIES OF TYPHOID, COLON, AND ALLIED
BACILLI.*

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INTRODUCTORY.

Until within the last few years the differentiation of the typhoid bacillus from the typical colon bacillus of Escherich and from the members of the group of allied bacilli — which may be briefly referred to as the Gärtner group — has depended upon physiological rather than upon morphological characters of the individual bacilli, or of their colonies. The similarity of the colony forms of these bacilli when grown in or upon the usual gelatin or agar media has led to endless difficulty in their recognition and separation when they occur simultaneously in material subjected to analysis. Indeed, this colony resemblance has probably been more largely responsible than any other resemblance for the origin of the belief in the close phylogenetic relationship and even interchangeability of these forms, which, as is well known, are really very distinct physiologically. Even members of the intermediate Gärtner group, especially the so-called “paracolon” or “paratyphoid” organisms, which approach the typhoid bacillus more closely physiologically than do the members of the typical colon group, are easily distinguished from the typhoid bacillus by their ability to ferment dextrose with the liberation of gas, as well as by their serum reactions.

The physiological relationship and the differentiation of the various members of these groups from one another has of late received much attention. This differentiation and

* Read March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, at Cleveland, Ohio.

identification has been largely based on the fermentative activities and agglutinating reactions, and the organisms, with the typhoid and colon bacilli representing the extremes, have thus been divided physiologically into various groups and sub-groups.

Some of these organisms undoubtedly represent just as permanent types as the typhoid and typical colon bacilli, while others, especially those distinguished simply by differences in agglutinating reaction, will probably be found to belong to one or other of the main types, from which they have been slightly specialized by a sojourn in some animal host. Briefly for purposes of reference, we may distinguish the following groups:

I. *The Typhoid Group*. — This group is represented by the typhoid bacillus, which does not ferment any carbohydrate with the evolution of *free* gas. Dextrose and some other saccharids — especially some monosaccharids — are fermented with the formation of acid. Probably the known organism which approaches the typhoid bacillus most closely physiologically, or rather fermentatively, is the *B. dysenteriae* of Shiga. The cultural appearances and fermentative reactions of both of these organisms are practically identical. Petruschky's *B. fecalis alkaligenes* approaches these organisms closely, but does not ferment any carbohydrate, either with the formation of acid or the production of gas.

II. *The Gärtner Group*. — Chief representative is Gärtner's *B. enteritidis*. The organisms of this group ferment dextrose with the formation of gas. They do not ferment lactose or saccharose either with the evolution of gas or the formation of acid. After primarily acidifying litmus whey they usually, sooner or later, give rise to an alkalization of the medium. In this group and its subdivisions may most conveniently be placed such organisms as "para colon" and "paratyphoid" bacilli, *B. typhi murium*, *B. psittacosis*, and *B. icteroides* (Sanarelli).

III. *The Colon Group*. — Represented by the (*a*) true *B. coli communis* of Escherich, which ferments dextrose and lactose with the evolution of gas, but does not ferment sac-

charose; and (b) by bacilli which ferment dextrose, lactose, and saccharose with the evolution of gas.

IV. *The Aërogenes Group*.—Represented by organisms probably related to the previous group which are, usually at least, not motile, but which ferment not only dextrose, lactose, and saccharosé, but also some polysaccharids with the evolution of gas.

We thus see that there is no difficulty in differentiating typhoid bacilli from typical colon bacilli by their physiological reactions, and further that there is no reason for confusing the typhoid bacillus with any of the more closely allied forms occurring in the Gärtner group when their physiology is carefully studied.*

With the individual and colony morphology, however, as above stated, it is different, and even the extreme types are not readily distinguished from each other by morphological characters.

Elsner's¹ modification of Holz's² acid potato medium, which he published in 1895, was the first contribution to the differentiation of the colonies of typhoid and colon bacilli, which was of practical value in their recognition and isolation. In 1895 Rosenthal³ had noted and studied colony variations in low percentage and semi-solid gelatin, and in 1896 Klie,⁴ continuing Rosenthal's experiments, called attention to spreading and thread-forming typhoid colonies in low percentage gelatin media. He also demonstrated the same appearances in colon colonies. Piorkowski⁵ recommended the use of urine gelatin for differentiating typhoid from colon colonies, and in 1899 proposed the use of a urine medium containing only three and three-tenths per cent gelatin, which was to be used at 22° C. This medium is practically Klie's medium, and suffers from the same disadvantage, namely, that colon bacilli at times form thready colonies in it as well as do the typhoid bacilli. Such media are practically useless on account of their absolute dependence for success upon temperature conditions which are diffi-

* On this subject see Cushing: Bull. Johns Hopkins Hospital, July-August, 1900, and Durham: Journ. Exp. Med., 1901, Vol. v, No. 4, p. 353.

cult to maintain. All gelatin media suffer not only from this disadvantage, which under certain conditions precludes their use in warm weather, but also from their liability, in practical isolation work, to become overgrown and fluidified by rapidly growing and peptonizing organisms.

A medium differing from all of these in being composed of agar two per cent, and which gives a differentiation of surface colonies of typhoid and colon bacilli, was devised and published by Capaldi⁶ in December, 1896. The surface typhoid colonies on this medium are flat, homogeneous, and lighter than the colon colonies, which are thicker and more granular. This medium has a practical value and can be used at all seasons of the year without difficulty, as the typical colonies are developed at 37° C.

Before the appearance of Klie's article I had noted differences in the action of typhoid and colon bacilli in semisolid media, and had already devised a medium in which typhoid and colon colonies could be distinguished from each other. This medium was at that time undergoing practical tests to determine its value in the isolation of typhoid bacilli from feces and urine. These tests appeared so satisfactory to us that the composition of the medium was finally published in 1897.⁷

It may for convenience be briefly stated here that the original medium used for the differentiation of colonies was composed of agar, ten grammes; gelatin, twenty-five grammes; Liebig's extract of meat, five grammes; sodium chloride, five grammes; and dextrose, ten grammes to one thousand cubic centimeters of distilled water. This medium was cleared by the use of the whites of two eggs, and had a final reaction of two per cent normal acid, phenolphthalin being the indicator. Normal hydrochloric acid was used in correcting.

Typhoid bacilli in plates from this medium form small light colonies of a greenish tinge by transmitted light with irregular outgrowths and fringing threads. Colon colonies are much larger, and, as a rule, are darker and do not have irregular outgrowths and fringing threads.

This medium is solid at 37° C., and the differentiation probably depends upon a tendency on the part of the typhoid bacilli to form threads in a medium of this acidity, especially when pepton is absent.

Besides this plating medium, the use of another medium, to which organisms from the thread-forming colonies were transferred, was recom-

mended. This medium is used for cultivation in tubes, and consists of agar, five grammes; gelatin, eighty grammes; Liebig's extract of meat, five grammes; sodium chloride, five grammes; dextrose, ten grammes; and distilled water, one thousand cubic centimeters. It is cleared with the whites of eggs and corrected to one and five-tenths per cent acidity, phenolphthalin being the indicator.

In a tube of this *semisolid* medium the growth of the typhoid bacillus produces uniform turbidity at 37° C., within eighteen hours. The colon cultures do not give uniform clouding, and present one of several appearances, dependent upon differences in degree of their motility, and upon their ability to form gas from the dextrose present in the medium.

Both of these media, the plating and the tube medium, are always used at 37° C., and when used as a check upon each other in the way indicated permit of the isolation, and, according to my experience, of the identification of the typhoid bacillus, the entire process not requiring over thirty-six hours. The practical results of this combined process have been elsewhere recorded, and show a large percentage of isolations of typhoid bacilli from feces in the series of cases investigated.⁸

Since the publication of the experiments on semisolid media, I have at intervals carried on further investigations on the colony differentiation of typhoid and colon bacilli. This has been done with the aim of devising a more easily prepared, and, if possible, a simpler medium than that originally recommended for the differentiation of the colonies of these bacilli.

The care required in the preparation, and the exact determination of the end reaction which is necessary to a successful development of characteristic colonies in that medium, has limited its application except in the hands of the more expert workers, and in the best equipped laboratories. The uncertainty attending the determination of end reactions with most of the indicators used in acidimetry and alkalimetry, and the necessity of having constantly on hand correct test solutions and accurate burettes, may well be urged, under certain circumstances, as grave objections to the use of media in which definite reactions have to obtain.

It has been my endeavor throughout these experiments to eliminate the necessity for titrating and correcting reactions. The experiments, therefore, recorded in this paper deal with a modification of the plating medium.

An ideal medium for this purpose of isolation and identification of typhoid bacilli would be one of agar or some

stiffening base that could be used at body temperature, in which the desired colonies would develop rapidly, and which would not become overgrown or fluidified by any rapidly growing or predominating organism; and further, a medium of simple composition, easily prepared, and not requiring the application of refined chemical methods and determinations in its preparation.

It is with the aim of offering at least a partial solution of this problem that these data are now published, and also because of the interest which seems to me to attach to the results of these experiments as demonstrations of what may sometimes be accomplished by the slightest divergence from routine procedures and traditional methods.

REMARKS ON THE COMPOSITION AND PREPARATION OF
'THE MEDIA DEvised FOR THE DIFFERENTIATION OF
THE COLONIES.

Agar is the principal stiffening base of all the media. It has always been dissolved in distilled water by vigorously boiling over a free flame for thirty to forty-five minutes. Other ingredients, such as Liebig's extract of meat, sodium, chloride, and gelatin were always added subsequent to the melting of the agar. Whenever sugar was added this was done after the medium had been cleared. Some of the media were cleared by simple filtration through paper; others by the addition of the whole or whites of two eggs to the liter, boiling for about thirty minutes, and filtering through absorbent cotton. Whenever titrations were made a one-twentieth NaOH solution was used, phenolphthalin being the indicator. Determinations were made on five cubic centimeters of the medium, boiled for three minutes in forty-five cubic centimeters of distilled water, and a fair pink color was taken as the end reaction. About ten cubic centimeters of the medium were put in each test tube, and these were sterilized in the usual manner at 100° C. on three successive days.

GENERAL DESCRIPTION OF THE COLONIES IN THE SPECIAL MEDIA.

Typhoid Colonies.—The *deep colonies*, constantly referred to in the text as typical or characteristic, are, among the typhoid, of irregular shape and have well-marked threads of bacilli given off from them. They vary in size according to the composition of the medium and the number of colonies in the plate. Their texture is generally loose, and their color often light green in the small colonies, becoming darker in the large ones. *Surface colonies* in the various media may be quite flat and homogeneous, or may show the denser center with threads spreading out from it. The deep colonies are often not more than one-fifth, and at times even less than one-tenth, as large as the deep colon colonies.

Colon Colonies.—The typical *deep* colon colonies are denser and darker as well as larger than the typhoid colonies, do not have irregular outgrowths and fringing threads, and are readily to be distinguished from typhoid colonies. The *surface colonies* in some of the media are quite characteristic, being large, disc-shaped, of a deep color nearly to the periphery, terminating in a well-marked light thin zone. This is especially true of those in the simpler agar media.

The foregoing description of course refers both to typhoid and colon colonies developed at 37° C. They are generally well developed and typical in eighteen hours, and certainly often in less, probably in twelve hours.

FACTORS WHICH INFLUENCE THE CHARACTERISTIC DEVELOPMENT OF COLONIES.

The cultures tested have usually been twentyfour-hour broth cultures of typhoid or colon bacilli, grown at 37° C., or mixtures of these made just previous to plating. Throughout my work on the isolation of typhoid bacilli from stools, urine, etc., it has seemed to me that the organisms present in these excretions acted in the isolating medium nearly as do the organisms from young broth cultures. Hence differentiations obtained when broth cultures are used will in most cases be

found to obtain among organisms in excreta; when old agar cultures are used the results are not so satisfactory.

One must also keep constantly in mind that the number of colonies developing in a given plate, pure or mixed, influences the morphology of the colonies. For instance, in nearly all cases the moderately crowded plates, especially the moderately crowded plates of pure cultures of typhoid bacilli, give the most characteristic colonies. In plates where the colonies are not numerous, apparently when the organisms are free from the influence of diffused metabolic products from the neighboring colonies or out of range of the extraction of food supply by these colonies, the typhoid colonies, unless the initial reaction of the medium is particularly favorable, do not develop characteristically; *i.e.*, are large, generally have only few or no threads, and take on the morphology looked for among the colon bacilli.

In mixed cultures, however, although the moderately crowded plates are the best, less crowded plates give clear differentiations, due probably to the greater changes brought about in the medium by the colon bacilli of nearby colonies.

The colon colonies, on the other hand, under the same circumstances as the typhoid, are very uniformly larger than the typhoid colonies, in mixed plates often five to ten times as large, and are of closer texture, darker, and generally of regular outline, sometimes with knob-like growths. In very crowded colonies there is in some cultures a tendency to irregularity of outline, and more rarely threads are formed.

These remarks apply to the action and appearance of colonies in all the media tested, and should be borne in mind in the practical application of these, either as differential or isolation media.

DESCRIPTION OF THE EXPERIMENTS ON COLONY DIFFERENTIATION.

The experiments in the order here given show a gradual evolution from the more complicated media which were a natural consequence of the theoretical considerations, and

few practical data upon which my earlier experiments were based, to the simpler media which have been found to favor differentiations. Only a few of the many combinations experimented with are given. With the aid of the general descriptions already given and reference to the photographs the following epitomized experiments should be intelligible.

Experiment I.—The medium* used in this experiment differs but slightly in composition from the original plating medium previously described. Its composition is as follows:

Agar	15 grammes.
Gelatin	15 "
Liebig's extract	5 "
NaCl	5 "
Dextrose	10 "
Distilled water	1,000 cubic centimeters.

This is cleared by the addition and coagulation of the whites of two eggs, and filtered through absorbent cotton. Its reaction is usually about one and two-tenths per cent acid, and no acid or alkali is added.

Test.—Platings of typhoid and colon bacilli in pure and mixed cultures were made and were examined after twenty hours at 37° C. The plates of pure typhoid were found to contain typical thread-forming colonies. The colon colonies were larger, and did not form threads. In the plates from mixed cultures the typhoid colonies developed well and were typical. (Plate XII., Figs. 1, 2, 3, and 4.)

This medium has been in practical use in our laboratory for two years, in experimental work in which the separation and estimation of the typhoid colonies is necessary, and has been found most satisfactory. Typhoid bacilli have also been isolated from feces by this medium in a number of cases (see below). The typhoid colonies are small, greenish, irregular, and fringed with threads.

Experiment II.—In this experiment no gelatin was used. The medium consists of:

* For the exact method and order of preparation see ante, p. 153.

Agar	15 grammes.
Liebig's extract	5 "
NaCl	5 "
Dextrose	10 "
Distilled water	1,000 cubic centimeters.

The reaction was found to be eight-tenths per cent acid and was not changed. The first specimen was filtered through paper.

Test. — Sixteen hours at 37° C. Typhoid colonies typical, well developed, and plentiful.

(a.) Tests of this medium with change of reaction to one per cent, one and two-tenths per cent, one and four-tenths per cent acid show that no colonies will develop in it at these degrees of acidity. With nine-tenths per cent acid most typhoid colonies were not typical. This shows that a high degree of acidity, not due to the addition of gelatin, is inimical to both typhoid and colon bacilli, but especially to typhoid bacilli.

(b.) This medium, tested with one cubic centimeter of normal NaOH solution added to the liter, gives, if anything, a better differentiation than without it.

(c.) The foregoing media in this experiment were filtered through paper and were not satisfactorily cleared in this way, hence a fresh specimen of this medium was prepared and cleared with the whites of two eggs to the liter and filtered through cotton. The slight increase in alkalinity from the addition of the eggs was, as seen from (b), favorable.

Test. — Eighteen hours at 37° C. Typhoid colonies abundant, well developed, and large majority typical. Colon colonies showed no threads. In plates from mixed cultures differentiation was good.

(d.) Same composition except that three grammes instead of five grammes of meat extract were used. Test shows colonies less numerous than in five-gramme medium, hence medium not so favorable. Differentiation good.

Experiment III. — Sodium chloride as well as gelatin was eliminated from this medium, which consists of:

Agar	15 grammes.
Liebig's extract	5 "
Dextrose	10 "
Distilled water	1,000 cubic centimeters.

Original reaction seventy-five one-hundredths per cent acid. No acid or alkali added. Cleared by the coagulation of the whites of two eggs, and filtered through absorbent cotton.

Test. — Twenty hours at 37° C. Typhoid colonies thread forming. Colon colonies no threads.

Experiment IV. — This medium was made of:

Agar	15 grammes.
Liebig's extract	5 "
NaCl	5 "
Distilled water	1,000 cubic centimeters.

The original reaction was found to be seventy-five one-hundredths per cent acid, and was not changed. The medium was cleared by the whites of two eggs to the liter and filtered through cotton.

Test. — Plates showed formation of threads nearly general in deep typhoid colonies. Surface colonies were flat, clear, and homogeneous. Large colonies at times slightly granular at center.

Mixed cultures, differentiation good. Typhoid colonies larger than in same medium plus dextrose. Colon colonies no threads. Surface colon colonies dark at center, light at periphery.

Experiment V. — As the different ingredients of the original medium were eliminated, or the combinations changed, it gradually became apparent that the differentiation was not absolutely dependent upon any of these with the exception, of course, of the meat extract, which was the nutrient base, and the agar, which was the stiffening ingredient of the media.

The simple combination of these was therefore tried:

Agar	15 grammes.
Liebig's extract	5 "
Distilled water	1,000 cubic centimeters.

The original reaction of this was seventy-five one-hundredths per cent acid, and was not modified by the addition of acid or alkali. The medium was cleared by the whites of two eggs and filtered through cotton.

Test. — Plates were examined after twenty hours at 37° C., and the differentiation was found to be excellent both in pure and mixed cultures, especially in the latter. (Plate XII. and XIII., Figs. 5, 6, 7, 8.)

CRITICAL REVIEW OF THE EXPERIMENTS.

These experiments show that any of these combinations give us a good basic medium for differential and isolation work with the typhoid bacillus, and that in most instances this is to be obtained without the correction of the original reaction of the medium; *i.e.*, addition of acid or alkali. At the same time some of the media would probably be a little more efficient with the reaction slightly altered. In testing feces and mixed cultures in media with or even without sugar, this necessary change of reaction is in many instances brought about by the metabolic changes wrought during the development of colon and other colonies. This is seen over and over again in the more marked type of the differentiation in mixed than in pure plates, which demonstrates to us that a medium of too feeble acidity to develop very typical colonies in pure culture often gives them in the mixed plates. This I have often noticed in my work on the isolation of typhoid bacilli from stools, and it led me to employ a medium of less acidity for this work than the one most suitable for the pure culture differentiation; for instance, a reduction of the original plating medium from a reaction of two per cent acidity to one and eight-tenths per cent or even less.

The most striking point, however, brought out by these experiments is the extreme simplicity of the medium which permits of the differentiation of the colony of typhoid ba-

cilli from that of the true *B. coli communis*, and from many, if not all, of the intermediate allied forms. We are also impressed with the fact that this simple medium which determines the formation of thready colonies is a solid medium, and of nearly the same composition as one of our most constantly used and familiar media—nutrient agar—simply lacking one important ingredient, pepton, and the unimportant ingredient sodium chloride. This fact would be of interest in itself even it had no practical bearing, since it demonstrates what may sometimes be accomplished by slight divergences from routine procedures, and also impresses us with the factors, some of them apparently unimportant, which may play a part in determining our idea of an organism and its relationships.

All of the other combinations tried—the most important of which alone have been given in the selected list of experiments—are practically unimportant variations of this simple basic medium composed of agar and meat extract. The different sugars, the sodium chloride, and gelatin are shown to be in no wise absolutely essential to the differentiation of the colonies, although their presence may under certain circumstances be of aid in bringing about the very best conditions for the development of characteristic colonies. The reduction of consistence due to the presence of the gelatin seems to have little or no effect on the formation of threads, and may be disregarded, the principal use of the gelatin, when added, being the determining effect of the increased acidity upon the thread formation of typhoid colonies. The colonies of typhoid bacilli are smaller in media to which gelatin has been added and the increased acidity not corrected. If the sugars are valuable at all it is as nutrient ingredients, or in giving rise to acids when used by the bacteria. This would be true only of dextrose in the case of typhoid bacilli, but of this and other sugars in plating mixed cultures in which members of the colon group are present. Organisms growing in the presence of these sugars give rise to acid products, thus raise the acidity of the medium and in that way bring more rapidly to the fore the tendency of the

typhoid bacilli to form irregular and thread-forming colonies.

The amount of agar used, whether one per cent or one and one-half per cent, is not very important: one and one-half per cent gives a stiffer and more satisfactory medium and tends to limit any outwandering of bacilli from the colonies.

PRACTICAL APPLICATION OF THE MEDIA.

(a.) *Application to the Isolation of Typhoid Bacilli.*—Any of the media described in the experiments, with slight modification of their initial reaction and in most instances without modification, will, with little doubt, be found applicable to practical work on the isolation of typhoid bacilli when occurring in the company of colon bacilli in materials such as feces. The medium, however, which has been most thoroughly tested in this regard is that composed of agar, fifteen grammes; gelatin, fifteen grammes; Liebig's extract of meat, five grammes; sodium chloride, five grammes; and dextrose, ten grammes; to one thousand cubic centimeters of distilled water. This is made according to the directions already given, and requires no correction of its reaction. It has been in constant use in our laboratory for nearly two years, not only in identifying typhoid colonies and making differential counts of their numbers in artificial mixtures of typhoid, colon, and various bacilli, which have been under observation in several experiments, but also in the isolation of typhoid bacilli from feces * and the cadaver.

Practical Suggestions for isolating Typhoid Bacilli from Feces.—Some practical directions and suggestions as to the best method of analyzing specimens of feces for the detection of typhoid bacilli may not be amiss here even at the risk of repeating what has already been said by me elsewhere on this subject.

The best method of making such an investigation is to transfer from one to several loopfuls of the feces to a tube of

* A very thorough test of the practical value of this medium in routine diagnostic work on typhoid feces has been made by Dr. H. A. Higley. See Higley: Medical News, March 29, 1902.

nutrient broth or sterile water, making a fairly cloudy emulsion. From this emulsion five or six plates are usually made by transferring one to five loopfuls of the emulsion to tubes containing the melted plate medium.* When the loopful of the emulsion is thoroughly mixed with the medium, this is poured into a Petri dish and allowed to harden. The plates are then placed in the incubator at 37° C., and allowed to remain for twelve to eighteen hours, when they are ready for examination.

If typical deep colonies with fringing threads and outgrowths are found the semisolid tube medium is then inoculated from them and placed in the incubator at 37° for from twelve to eighteen hours. Besides these typical deep colonies, flat homogeneous colorless surface colonies, or flat colonies with a thread-forming colony at the center, are very typical of the growth of the typhoid bacillus in the plating medium we have just described, and in fact in the simplest of these new media. These colonies with a little practice may readily be distinguished from the surface colonies, which as a rule are larger, denser at the center, with a distinctly lighter peripheral line, are yellow in color, and are apt to be granular. (Plate XIII., Fig. 8.)

I have several times been impressed with the fact, when examining plates from feces or mixtures, that when the deep colonies are plentiful and typical, surface colonies are rare; but that when the deep colonies are not to be made out or are not typical, surface colonies abound. The medium thus offers a better chance for the detection of typhoid colonies than does the original medium, in which surface typhoid colonies were seldom in evidence. Such surface colonies are also to be tested by transplanting to the semisolid tube medium, and the identity of the organisms comprising them thus further established.

The typical thread-forming colonies, taken in connection with the diffuse clouding and non-gas formation in the tube medium are found so seldom, if indeed ever, as characters

* Care should be taken to ascertain that this medium has been thoroughly melted, otherwise the colonies will not develop typically in the plates made from it.

of organisms other than typhoid bacilli occurring in feces or other materials subjected to investigation for typhoid bacilli, that our experience indicates that the typhoid bacillus may be recognized by these two characters, and a diagnosis thus made in from twenty-four to thirty-six hours. Organisms suspected of being typhoid bacilli from displaying these characters have always been found upon subsequent examination to correspond in all their reactions and characters to typical typhoid bacilli.

(b.) *Application to the Differentiation of Typhoid Bacilli from Various Allied Forms.*— By a combined use of the medium for colony differentiation and the medium for differentiation of pure cultures in tube cultivations,* typhoid bacilli may be differentiated in pure culture, not only from true colon bacilli, but also from the bacilli forming the intermediate groups.

As has been shown in a preceding section, the colonies of typhoid bacilli are to be distinguished from those of colon bacilli in the several media experimented with, by the formation of irregular colonies fringed with threads; the colon colonies remaining more regular and without threads. In the semisolid medium used in tubes the typhoid bacilli develop rapidly and spread throughout the medium, thus giving rise to a more or less extended uniform clouding, but no gas is produced.

The colon colonies ferment the glucose of this medium with the evolution of gas, and may or may not spread widely from the line of inoculation and thus cloud the medium, or leave it clear according to the activity of their motility. In some instances gas is not at first liberated spontaneously, and no bubbles are apparent; but if the medium is stirred with a platinum needle, bubbles develop in its track, and the culture can readily be distinguished from one of typhoid, in which no bubbles will develop when it is thus stirred.

Practically the same conditions obtain in testing organisms of the intermediate or Gärtner group. Several cultures of this group have been studied, among them *B. enteritidis*, *B.*

* See *ante*.

psittacosis, *B. icteroïdes* (Sanarelli), *B. typhi murium*, *B. paracolon* (Gwyn), *Bacillus O.* (Cushing), and *B. paratyphoid* "Seemann" (Schottmüller), and *B. paratyphoid* "Müller" (Schottmüller),* and an unidentified organism belonging to this group, isolated by Dr. Charles Norris, from the spleen of a patient dying with symptoms of septicemia. *Bacillus enteritidis* is the only one of these organisms which shows a marked tendency to irregularity of colonies and to thread formation in the plating media. Its colonies sometimes cannot be distinguished from those of the typhoid bacillus. This fact is of interest, as it suggests the possibility of distinguishing various members of the Gärtner group from each other by differences in colony appearances, as brought out in such media as those used in these experiments. More can undoubtedly be expected from a careful investigation of these colony variations than has been developed by our rather cursory observations. The difficulty with which several of these bacilli are differentiated and identified by the usual fermentation, animal, and serum tests make such morphological characters of recognition most desirable.

As has just been said, all members of this group with the exception of *B. enteritidis* are to be readily distinguished from typhoid bacilli by the differences of their colonies; and little difficulty is experienced in recognizing typhoid colonies in mixed cultures containing these organisms. Even if doubt exists in regard to any given colony, a test of the organisms in the tube medium would readily distinguish any member of this group from typhoid bacilli, since all members of the Gärtner group ferment glucose with the evolution of free gas, and are thus readily to be distinguished from *B. typhosus*, even though they cloud the medium.

Of the organisms more closely approaching typhoid bacilli in their fermentative and cultural characters, I can only speak from personal observation of one species — *B. dysenteriae*. With *B. fecalis alkaligenes* I have made no experiments.†

* Transplants of these two organisms were given to me by Dr. Norris who had recently obtained them through the courtesy of Dr. Schottmüller.

† A culture marked *B. fecalis alkaligenes*, received from Král's laboratory, and whose original source I do not know, seems to me to undoubtedly belong to the non-liquefying fluorescent bacilli, as it develops a faint greenish soluble pigment.

In regard to the dysentery bacillus it seems of interest to note that two cultures of this organism, known respectively as "Kruse" and "Flexner," kindly sent to me by Prof. Simon Flexner, could be differentiated from typhoid bacilli in the media under discussion. This organism, as is well known, does not give rise to gas in the presence of any of the sugars, but in cultural characters very closely resembles typhoid bacilli. In the simpler agar plate medium above described, its colonies with a few rare exceptions were of regular outline and did not form threads. The colonies, however, showed great discrepancy in size, some being very large and dark, others very small and light green. This difference in the size of colonies is not marked either among typhoid or colon bacilli. This peculiarity was shown by both of the cultures of *B. dysenteriae* investigated.

In the semisolid tube medium the dysentery cultures of course do not give rise to gas, and, which is of more interest, do not spread out through the medium from the line of inoculation. This readily distinguishes them from typhoid bacilli. Cultures of this bacillus are, however, by some observers said to be slightly motile when first isolated, and it is, therefore, possible that when first coming from the human or animal body they may cloud the medium. These organisms were found by me not to develop in as acid a medium as that in which typhoid bacilli will grow.

Summary.

Typhoid bacilli, as is well known, can be differentiated without difficulty physiologically from typical colon bacilli and most allied forms. In the usual media containing pepton which are used for plating, nutrient agar and nutrient gelatin colonies of these bacilli are so similar that they are not to be distinguished from each other. This has been a serious hindrance to the proper investigation and bacteriological diagnosis of typhoid fever.

Many attempts, which have met with only partial success, have been made to devise media which will serve for the practical differentiation and recognition of these colonies.

Most of these media are of limited application and are uncertain in their differentiation, or are too difficult of preparation for general use.

The experiments forming the subject of the present paper show that by the use of such a simple medium as one composed of agar, Liebig's extract of meat, and distilled water, or by media differing slightly from this by the addition of gelatin, sugar, or salt, or a combination of these, it is possible to readily differentiate in original plates the colonies of typhoid bacilli from those of colon and allied bacilli.

One of these media (see Experiment I.) has been thoroughly tested practically and successfully in the isolation of typhoid bacilli from the excreta of typhoid fever patients.

These media being composed entirely or mainly of agar are uninfluenced by fluidifying organisms. They are used at 37° C., and typhoid bacilli develop typical colonies in them in from twelve to eighteen hours.

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DESCRIPTION OF PLATES

ILLUSTRATING THE DIFFERENTIATION OF TYPHOID AND COLON COLONIES.

PLATE XII.

FIG. 1. — Photograph of deep colonies of typhoid bacilli, showing fringing threads. (See Exp. I.) Grown for eighteen hours at 37° C. in a medium composed of agar, fifteen grammes, gelatin, fifteen grammes, Liebig's extract of meat, five grammes, NaCl, five grammes, dextrose, ten grammes, and distilled water, one thousand cubic centimeters.

FIG. 2. — Photograph of deep colonies of colon bacilli, demonstrating the absence of thread formation. Grown for eighteen hours at 37° C., in the same medium as that described under Figure 1.

FIG. 3. — Photograph of deep typhoid and colon colonies from a mixed culture of typhoid and colon bacilli. Grown for eighteen hours at 37° C., in the medium described under Figure 1. The typhoid colony is small and has threads growing out from it. The colon colony is large and without threads.

FIG. 4. — Deep typhoid colony grown for eighteen hours at 37° C., in the medium described under Figure 1. High magnification.

FIG. 5. — Photograph of deep colonies of typhoid bacilli, showing fringing threads. Grown for eighteen hours at 37° C., in a medium composed of agar, fifteen grammes, Liebig's extract of meat, five grammes, and distilled water, one thousand cubic centimeters (See Exp. V.).

PLATE XIII.

FIG. 6. — Photograph of deep colonies of colon bacilli, demonstrating the absence of thread formation. Grown for eighteen hours at 37° C., in the agar medium described under Figure 5. The magnification is the same as in Figure 5, but the colonies are small on account of the greater number in the culture plate.

FIG. 7. — Photograph of deep typhoid and colon colonies from a mixed culture of typhoid and colon bacilli. Grown for eighteen hours at 37° C., in the agar medium described under Figure 5. The typhoid colony is small, of loose texture, and has fringing threads. The colon colony is large and without threads.

FIG. 8. — Photograph of surface typhoid and colon colonies from a mixed culture of typhoid and colon bacilli. Grown for eighteen hours at 37° C., in the medium described under Figure 5. The typhoid colony is thin, transparent, and homogeneous. The colon colony is larger, thicker, granular, and darker.

ON THE BACTERIOLOGIC STUDY OF A CASE OF PARACOLON
INFECTION PROBABLY SECONDARY TO TYPHOID FEVER,
WITH REMARKS ON SERUM REACTIONS IN PARACOLON
INFECTIONS AND ON THE THREAD REACTION.*

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NOTES ON THE HISTORY OF THE SUBJECT.

The subject of infections by members of the group of organisms intermediate to the typhoid bacillus and the typical colon bacillus of Escherich—the so-called paracolon bacilli—is one of ever-increasing interest. The name “paracolon” was first applied to the group by Gilbert,¹ who described five types of paracolon bacilli. He found members of this group present in the intestinal canal of man and many animals.

The first important cases † of infection by such organisms were described by Achard and Bensaude² in 1896, under the title of “Paratyphoid Infections.” Their cases were as follows:

Case I.—A case clinically resembling typhoid fever with an intercurrent relapse followed by a femoral phlebitis. During the course of the patient's illness the writers twice isolated from the urine, which had become purulent, a so-called paratyphoid bacillus which was identical with the one found in the second case. A blood culture made during the height of the fever proved negative. Aspiration of the spleen revealed staphylococci only. The stools contained only the usual forms of colon bacilli, typhoid bacilli being absent. The patient recovered after an illness lasting six weeks. During the course of the disease the authors obtained a positive serum reaction with the paratyphoid bacillus and with

* Read, March 28, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, Ohio.

† In this summary of the cases we shall exclude the epidemics of meat poisoning and the cases of psittacosis.

several cultures of typhoid bacilli, the reaction with the typhoid bacillus persisting for less than two months after defervescence.

The authors bring up the question whether or not the case was one of typhoid fever, complicated by a pyelonephritis due to the paratyphoid bacillus, and cite a number of cases in which colon bacilli had been found in cases of cystitis and pyelonephritis complicating typhoid fever. They conclude that the illness was due to the paratyphoid bacillus alone because the serum of animals which had been inoculated with their paratyphoid bacillus agglutinated not only the paratyphoid bacillus, but also some varieties of the typhoid bacillus. It is unnecessary to enter into any prolonged discussion of the subject, because, as Widal and Nobecourt³ very properly remark, the serum reactions in this case are of no value as the authors did not make use of any dilutions of the serum.

Case II.—The second case was that of a female infant aged seven months, which, after a febrile illness of almost three weeks' duration, beginning with a bronchitis, developed a suppuration of the right sterno-clavicular joint. From the pus an organism was obtained resembling that found in the first case. Both organisms closely resembled the *Bacillus psittacosis*. No other examinations were made in the case, and, as the authors state, typhoid fever could not be excluded. The description of these organisms will be detailed later in connection with the discussion of the bacillus isolated from our own case.

The next case to be reported was that of Widal and Nobecourt³ in 1897. A phthisical patient, after being three weeks in the ward, developed an abscess in the neck near the esophagus, with slight constitutional symptoms. From the pus a paracolon bacillus was isolated. (This case is considered by some authors as if it were the sequela of an attack of typhoid fever, but the patient at the time of observation was thirty-one years old and the attack of typhoid fever occurred at the age of nine.)

Widal and Nobecourt state that they are opposed to the term "paratyphoid" because the organisms more closely

resemble the colon bacillus than the typhoid bacillus. They cite some observations of Tavel and Lanz,⁴ who found similar organisms in cases of peritonitis, and they mention a case of suppuration in the spleen due to a related organism.

In 1898 Gwyn⁵ reported the case of a man who suffered from a typical attack of typhoid fever with intestinal hemorrhages. The Widal reaction was absent. Three and a half weeks after the onset of the disease Gwyn isolated from the blood a paracolon bacillus.

Malherbe and Mounier⁶ isolated a paracolon bacillus from a case of inflammation of the penis, with induration of the corpus cavernosum.

Cushing's⁷ case is the most complete study among the various papers on the subject. The history was in brief the following:

A man twenty-seven years of age suffered for ten weeks with an attack of fever described as typhoid. He had a relapse beginning with an epistaxis. During convalescence he developed two foci of costochondral osteomyelitis. One of the foci disappeared, but the other broke open six months later and discharged pus. Since that time he had a sinus at the level of the fifth rib at the edge of the sternum. From this focus, nine months after the onset of the fever, Cushing obtained the paracolon bacillus in pure culture. The Widal reaction was negative.

Cushing refers to the report of a case by Blumer⁸ in which the latter stated that he found the bacterium coli in a post-typhoidal rib abscess. As the organism produced alkali in milk and therefore probably did not ferment lactose, Cushing believed that it was probably a member of the group of bacilli under discussion.

Schottmueller⁹ in 1900 reported a case of fever lasting twelve days, beginning with reddening of the conjunctiva and the nasal mucous membrane. A roseola and enlarged spleen were present. The Widal reaction was absent. The blood culture made at the height of the attack yielded a "typhoid-like" bacillus. Later¹⁰ he reported five cases occurring during a small epidemic of typhoid fever, in all of

which the Widal reaction was absent and from the blood of each of which he succeeded in isolating "typhoid-like" bacilli. The clinical picture resembled typhoid fever. All of the patients recovered; there were no complications and no relapses. (So the author states; but his second case certainly must be looked upon as having had a slight relapse.)

The report of only one Widal reaction is made. The blood of each of the patients gave a positive reaction with the organism isolated from the case and with some of those isolated from the other cases, in high dilutions. The complete data will be given later. In the sixth case he made the diagnosis on the basis of the serum reactions alone.

Kurth¹¹ in a series of sixty-two cases of typhoid fever found five which did not give a positive Widal reaction. From the urine of one case and the stools of another, he isolated a bacillus probably belonging to the group under discussion, with which a positive reaction was obtained with the serum of four of the cases. (The fifth was the case with the bacillus in the urine; no serum was obtained from this case.)

Although it is not my purpose to enter into the description of the cases clinically in this paper, it is essential to refer to a few facts concerning the cases of Kurth and Schottmueller. In five of Schottmueller's seven cases the duration was respectively twelve days, nineteen days, seventeen days, sixteen days, and thirteen days. In one the onset was characterized by headache and sneezing; in three by reddening of the face, conjunctivæ, and the nasal mucous membrane. In four of the cases there was constipation; in one diarrhea. In one case the roseola developed by the fourth day. All of the cases ended by lysis.

Kurth's cases lasted respectively three weeks, fifteen days, twelve days, eighteen days, and two weeks. All had diarrhea. In one case the roseola was present on the sixth day and in one on the fifth day. In one case the temperature dropped by a distinct crisis.

These data are given to emphasize the mention of a paper

published by Brill¹² in 1898 before Gwyn and Cushing reported their results. Brill reported a series of cases, which, though they were usually considered instances of typhoid fever, he believed should be placed in a distinct group, and which he believed could be diagnosed by their clinical appearances and the absence of the Widal reaction during the duration of the fever and during convalescence. In none of Brill's cases could the typhoid bacillus be isolated from the feces; in one, a variety of the colon bacillus was isolated from the blood aspirated from the spleen. (The cultural characteristics are not detailed.) Some of Schottmueller's and Kurth's cases resembled the clinical picture described by Brill.

Because of the absence of indican, acetone, and diacetic acid from the urine, Brill was not inclined to assume that the condition was due to a toxic agent produced in the intestinal canal; he suspected that the cases might be due to an infection by colon bacilli. The investigation of his own cases, as far as it went, did not seem to confirm this view sufficiently.

The last case to be reported was one presented by Buxton and Coleman¹³ to the New York Pathological Society, in February of this year. Clinically it resembled typhoid fever, and the paracolon bacillus was isolated from the blood. The Widal reaction was practically negative, but a reaction was present with the paracolon bacillus. All the cases thus far referred to recovered.

The clinical history of our case will not be given in full, because it is to be made the subject of a special article by Dr. Berg and the writer.¹⁴

HISTORY OF OUR CASE.

C. I., thirty-three years of age, admitted to the Mt. Sinai Hospital, on August 31, 1901. Previous history practically negative. Present history: Ten days ago the patient was suddenly taken ill with nausea, vomiting, and prostration. Temperature, 100° by mouth; there was abdominal distension and pain in the epigastrium. He remained in bed only two days, but was too weak to return to his work. Three days before admission he was seized with severe colicky pain located in the epigastrium and

right hypochondrium, vomiting and constipation; his skin was subicteric. Temperature, 103° F. On admission the liver was not found enlarged, but there were friction râles heard over it. Below the costal margin there was felt a very tender mass about the size of a large lemon. Spleen enlarged to percussion. No roseola present. Leucocytes, fifteen thousand. Ehrlich reaction not present. On the day after admission Dr. Berg performed an exploratory laparotomy, suspecting either a cholecystitis or an abscess of the liver. The mass felt was found to be a Riedel lobe. The gall-bladder was distended, its contents could not be expressed. There was fibrin over the liver. The gall-bladder was aspirated and dark thick bile obtained. After the operation the temperature varied between 103° – 106° F., intense jaundice was established with marked cerebral symptoms. Incessant vomiting developed on September 9, and the patient died on the following day. There was an agonal polymorphonuclear leucocytosis.

BACTERIOLOGIC EXAMINATION.

On September first, the fluid aspirated from the gall-bladder was plated, and a pure culture of an organism identical with that found later in the blood and urine was found. On the following day I made a blood-culture to determine whether the presence of the bacilli in the gall-bladder might not be due to an excretion of organisms from the blood. Eleven cubic centimeters of blood were used, part being plated and part being grown in fluid media. From both sources was obtained a pure growth of a paracolon organism.

On September 8 the urine was plated and a large number of colonies of the paracolon bacillus was found with a few colonies of the staphylococcus albus.

On September 9, about twelve hours ante-mortem, another blood-culture was made, eleven cubic centimeters again being used. There were obtained numerous colonies of a paracolon organism and a few colonies of the staphylococcus albus and streptococci.

A few hours after the fatal termination a culture was made from the spleen. There were found colonies of the paracolon bacillus associated with the staphylococcus citreus and albus and streptococci. From the blood aspirated from the heart the same organisms were recovered as were found in the blood the day before death. (The staphylococcus albus and streptococci probably represent an agonal invasion.)

A culture of the contents of the ileum, made at the time of the post-mortem examination, revealed two forms of colon bacilli (both of which coagulated milk and produced indol), the *Bacterium proteus vulgare*, *Staphylococcus albus*, and *Streptococci*. There were no typhoid bacilli and no paracolon bacilli found. For all these investigations, serum-glucose media were used.

DESCRIPTION OF THE PARACOLON BACILLUS.

(All media sugar free, except when otherwise mentioned.)

Titre of media one per cent acid (with phenolphthalein).

The organism is a facultative anaerobe.

Morphology. — The organism resembles the typhoid bacillus in morphology. In glucose-agar, after twenty-four hours, it appears in larger forms, and after forty-eight hours shows some involution forms and vacuolization. After two days on potato it shows rather distinct bipolar staining. No capsules; no spores. It stains like the typhoid and colon bacillus, being decolorized by the Gram procedure. The organism is markedly motile in bouillon cultures at 35° C. and 37° C., and retains its motility for several days. Six to twelve long flagella are present, arranged in peritrichal fashion. In gelatin and agar, surface and stick cultures, and in glucose-agar (surface) it resembles the typhoid bacillus, but the growth is more profuse and whiter in color. Gelatin is not fluidified. Agar plates emit an odor resembling that of the colon bacillus. Macroscopically, the surface colonies are somewhat larger than those of the *B. typhosus* and whiter. Microscopically, the smaller colonies show a densely granular center, the colony becoming less and less granular toward the periphery. The larger colonies have a brown irregular nucleus which is coarsely granular; the remainder of the colony is made up of heaps of granules which are browner near the center and larger in the clearer area at the periphery. The margin of a colony is round or irregular. The deep colonies are brownish in color, oval or whetstone-shaped; irregularly shaped knobs are often present.

Bouillon. — Diffusely clouded in twenty-four hours. Turbidity intermediate between the *B. typhosus* and *B. coli*; white, fine, powdery sediment. After forty-eight hours there is a marked fluffy deposit. There is no scum formed even after seventy-two hours. The cultures emit an odor like cultures of *B. coli*, but less marked.

Reaction with Carbohydrates. — (One-half per cent sugars used.) Glucose is split up with the production of acid and visible gas. In glucose-bouillon one and five-tenths per

cent acid produced in six days. In fermentation tubes maximum gas produced in twenty-four hours, amounting to thirty-three one-hundredths per cent (Einhorn tube). Maltose is split up with the production of acid and visible gas. Maximum acid one and twenty-five one-hundredths per cent; maximum gas after two days, fifty one-hundredths per cent. Lactose and sucrose are not affected. In fermentation tubes there is no growth in the branch; in the bulb there is active growth with alkali production. Amount of alkali produced same as in ordinary bouillon, being one and five-tenths per cent after one month.

Corresponding to its effect in the fluid media, the organism causes precipitation and gas production in two per cent maltose and two per cent glucose-serum-agar, but none in the corresponding lactose and sucrose media.¹⁵

Plain Milk in Tubes.—Unchanged for several days, then becomes yellowish and more and more translucent, the change being very marked after four weeks. The fat then floats on the surface and a white deposit is seen at the bottom of the tube. Boiling causes no coagulation.

Plain Milk in Fermentation Tubes.—In the branch a bubble of gas, otherwise unchanged. Bulb same as milk tubes.

Litmus Milk in Tubes.—After twenty-four hours distinct though slight acid reaction. After a few days (varying from three to six) reduction, and then quite marked progressively increasing alkali production. (Tubes often again show reduction at the bottom after three weeks.)

Litmus Milk in Fermentation Tubes.—After twenty-four hours bulb and branch slightly acid, bubble of gas in branch. After forty-eight hours, reduction in branch; later marked alkali production in bulb; the branch becomes blank white in color. Milk is never coagulated.

Potato.—A slight white shining growth is the rule. On the same potato-tubes the typhoid bacillus gives no visible growth. (Occasionally a moderate yellowish-green growth was obtained with our organism.)

Indol Reaction.—Positive but slight in peptone water and

sugar-free broth, tested after eight days with sulphuric acid and one ten-thousandth sodium nitrite. In some tests made in peptone water with one-half of one per cent sodium nitrite, a positive result was obtained with our paracolon bacillus after three days, with the colon bacillus in twenty-four hours, but was absent with the typhoid bacillus even after eight days.

PATHOGENESIS.

The organism was pathogenic for guinea-pigs and rabbits, producing peritonitis when inoculated intraperitoneally, and otherwise causing septicemia with degeneration of the viscera and focal necroses in the liver.

PROTOCOL OF ANIMAL EXPERIMENTS.

(In all experiments twenty-four-hour bouillon cultures used.)

Guinea-pig 1. — One cubic centimeter of culture of bacillus from the blood, intraperitoneally. Death in sixteen hours. Sero-purulent fluid in the abdomen; fresh fibrin on intestines and liver. Organism recovered from peritoneum and heart blood.

Guinea-pig 2. — One cubic centimeter of culture of bacillus found in second blood-culture, intraperitoneally. Died after fifteen hours. The result as above.

Guinea-pig 3. — The same experiment with bacillus from gall-bladder. Same result.

Guinea-pigs 4 and 5. — Same experiment with paracolon organisms obtained from the urine. Result practically the same.

Guinea-pig 6. — One cubic centimeter of culture of bacillus isolated from the blood, intraperitoneally. Death after eighteen hours. Cloudy serous exudate with fibrin in peritoneal cavity, a few hemorrhages in mesentery. Organism recovered. Liver shows parenchymatous degeneration.

In the succeeding experiments the organism used was the paracolon bacillus isolated in the first blood-culture.

Guinea-pig 7. — One cubic centimeter subcutaneously. Death after eight days. There was an indurated area at the site of injection not extending to the muscles or peritoneum. The peritoneum was injected. There was a slight non-purulent exudate. The intestines showed marked injection. The liver showed focal necroses with infiltration by leucocytes. There were also small areas in which there was a collection of round cells and polynuclear leucocytes. In one of the portal vessels a bacterial thrombus was found. Lungs congested and contain small hemorrhagic areas. Spleen

shows areas of suppuration; kidneys, parenchymatous degeneration and congestion. Organism recovered from indurated area and heart blood.

Guinea-pig 8. — Intraperitoneal inoculation with one-fourth cubic centimeter. Died after fifteen hours. Result same as with guinea-pigs 1 and 2.

Guinea-pig 9. — One-eighth cubic centimeter intraperitoneal inoculation. Died after thirty-six hours. Sero-purulent peritonitis, moderate pericardial effusion. Liver, parenchymatous degeneration with hemorrhages under the capsule. Kidney, parenchymatous degeneration. Organism recovered from peritoneum, pericardium, and heart blood.

Rabbit 1. — Two cubic centimeters; intravenous. No result.

Rabbit 2. — Two cubic centimeters; intravenous. Death after sixty hours. Liver shows parenchymatous degeneration; also foci consisting of round cells and polynuclear cells located mainly around the portal vessels, hemorrhage under the capsule and focal necroses. Kidney, parenchymatous degeneration, occasional purulent focus. Organism recovered from heart blood.

NOTES ON THE POST-MORTEM EXAMINATION OF OUR CASE.

It was only with the greatest difficulty that permission for an autopsy was obtained. We were restricted to an examination through the incision in the right hypochondrium, which had not entirely healed. The examination was made twenty-one hours post-mortem, and meanwhile the body had been embalmed, formalin being used. We were under the continuous surveillance of a relative. As a result, we can give only an incomplete report. Enough material was obtained, however, to make a fairly satisfactory microscopic examination. The results of the macroscopic and microscopic examinations will be combined in this report. All the organs were bile-stained.

Lungs. — Fibrin over both upper lobes.

Heart. — (The changes in the valves which were found and which explained a murmur heard during life were apparently dependent upon a traumatism sustained in early childhood. They are irrelevant here. We hope to report on this aspect of the case at some other time.) Parenchymatous myocarditis; many of the nuclei are very large, and are irregular in shape; fragmentation of the muscle-fibers.

Spleen. — Slightly enlarged (evidently was very large, but was shrunken by the preservative). Fibrin on the surface. Microscopically there are the changes of chronic interstitial splenitis and acute hyperplastic splenitis. The capsule is very thick; the trabeculæ and the walls of the arteries likewise. There is intense congestion of the pulp and marked pigmentation. There is also a marked proliferation of endothelioid cells, and a number of phagocytic cells, most of the latter containing pigment granules. The endothelium of the smaller vessels is markedly swollen.

Scattered through the pulp are a number of large clumps of bacilli. Only at the margins of the clumps can individual bacilli be seen. The bacilli resemble the paracolon bacillus in morphology and are decolorized by the Gram procedure.

Liver. — Moderately enlarged; fibrin on surface. Microscopically there are present the lesions of advanced chronic congestion. The capsule is thickened. There is marked parenchymatous degeneration present; this being more marked near the center of the lobules. About the branches of the portal vein there is a slight increase in the connective tissue, and an accumulation of lymphoid and epithelioid cells. The endothelium of the vessels is swollen, and an occasional phagocyte is present in the branches of the portal vein. The organ is markedly pigmented. The bile-ducts show no changes.

Gall-bladder. — Wall shows no changes.

Pancreas. — Slight grade of interstitial pancreatitis; islands of Langerhans not involved. The intima of the arteries is thickened.

Kidneys. — Normal in size; capsules adherent. Microscopically there is marked congestion, parenchymatous degeneration and pigmentation. The arteries show marked thickening of the intima, and there are some areas of chronic interstitial changes. The cells lining Bowman's capsules are swollen and there is a marked hyperplasia of the cells covering the glomerular tufts. Some of the tubules contain casts.

Intestines. — On the surface of the coils of a small intestine were the slight remains of a fibrinous peritonitis. Only the lower ileum could be examined. Scattered throughout it are a number of depressed, pale, round or oval areas. These vary in size from those two by one centimeter to those three by four centimeters. They are thinner than the surrounding part of the intestine and are mostly oval in shape, the smaller diameter lying transversely.

These appear to be healed ulcers. This view is confirmed by finding several similar areas where the submucosa is still exposed in the center of the areas.

Microscopical examination shows that the changes found are of recent date, for there are marked inflammatory changes present. Some of the Peyer's patches have evidently been partially ulcerated away, and are covered by a layer of tissue containing round and epithelioid cells. The part of the patch remaining shows hyperplastic changes. Some of the lymphoid nodules are apparently normal. The areas which appear to have been the site of ulcerations are, for the most part, not covered by villi. Between the tubules, near the sites of the healed ulcers, there is a marked collection of lymphoid and epithelioid cells.

SERUM REACTIONS IN OUR CASE.

On August 31st, September 1st, and September 2d the Widal reaction was negative in a dilution of one to twenty.

September 3d. — Serum gave no Widal reaction. With the bacillus isolated from the gall-bladder, there was a thread reaction (see below) in a dilution of one to thirty. This appeared after three hours and was present after twenty-four hours. In a dilution of one to one hundred there were threads in three hours, followed by an agglutination reaction in twenty-four hours.

September 7th. — Serum from a typhoid case with typhoid bacilli one to two hundred; positive instantaneously. Serum from same typhoid case with the paracolon bacillus one to twenty; negative. Serum Ireland * + typhoid bacilli one to two hundred, positive; one to five hundred, negative. Serum Ireland + bacillus from blood, thread reaction one to one hundred; after twenty-four hours, some clumps. Serum Ireland + bacillus from gall-bladder, one to twenty, threads; after twenty-four hours bacteria, partially clumped, partially in threads.

September 8th. — Serum Ireland + bacillus from gall-bladder one to twenty; a few threads after three hours; after twenty-four hours, negative. Serum Ireland with bacillus from blood one to fifty, thread reaction after three hours; persists after twenty-four hours. Serum Ireland with typhoid bacillus one to two hundred and fifty, positive. The serum of two cases of typhoid fever, two cases of appendicitis, and two cases of gall-bladder disease (the latter four being due to colon bacilli) gave no reaction with our organism.

September 9th. — Serum Ireland with typhoid bacilli one to two hundred; positive. Serum Ireland with bacillus from gall-bladder one to twenty, as on September 8th. Serum Ireland with bacillus from blood one to twenty and one to fifty, gave thread reaction in three hours; clumps after twenty-four hours. Serum from three cases of typhoid fever which reacted positively to the typhoid bacillus, gave no reaction in dilution of one to twenty with our organism.

September 10th. — Serum Ireland gave same results as before with the bacillus from the blood, the bacillus from the gall-bladder, and the typhoid bacillus, but gave no reaction with two varieties of colon bacilli from the stools of another patient.

September 12th. — The serum gave a thread reaction in three to four hours, followed by partial or complete agglutination in a dilution of one to twenty with the bacilli isolated from the blood, urine, and the gall-bladder. With the bacillus isolated from the second blood-culture reaction was positive in a dilution of one to fifty. Serum Ireland gave no reaction with a colon bacillus from a case of appendicitis and one from a case of cholecystitis.

* Serum Ireland is serum from our case.

September 13th. — Serum gave a positive reaction in dilution of one to fifty with the bacillus isolated from the spleen post-mortem, but gave absolutely no reaction with the colon bacilli isolated from the intestine.

September 16th. — Reactions were made in dilutions of one to twenty only. With all the paracolon bacilli isolated from the case there was a thread reaction after three to four hours, followed in twenty-four hours by an agglutination reaction, or by a reaction consisting partly of clumps and partly of threads. The serum gave no reaction with the bacilli isolated from the stools and reacted positively with the typhoid bacillus.

Summary.

It will thus be seen that the patient's blood gave a specific reaction with both the typhoid bacillus and the bacillus isolated from the case. Further notes on these reactions will be given later.

On March 7, 1902, the serum still gave a positive reaction with the typhoid bacillus, but gave no reaction even in a dilution of one to five with the paracolon bacillus isolated from our case, the bacillus of Gwyn, the bacillus "O" of Cushing, two varieties ("Mueller" and "Seeman") of Schottmueller's bacilli, Buxton's bacillus, a paracolon bacillus isolated by Dr. Norris, and an organism of the hog-cholera group isolated by Dr. Lartigau.

RELATIONSHIP OF OUR ORGANISM TO OTHER SIMILAR ORGANISMS, WITH NOTES ON THE LATTER.

Because of the incomplete description given by some of the writers, it is difficult to group their bacilli accurately. Furthermore, variations in the media used by them interfere to a great extent in comparing the results.

Durham¹⁶ originally placed in the "intermediate group" a number of organisms concerned in the etiology of meat poisoning. Later Cushing⁷ studied the *B. enteritidis*, the *B. morbificans bovis* of Basenau, the *B. Hatton* of Durham, the *B. cholerae suis*, the *B. icteroïdes* of Sanarelli, and the *B. typhi murium* of Löffler. He finds that all these organisms correspond in their main features, which are the following:

They are identical in morphology with the typhoid bacillus; are motile, having as many or more flagella than the

typhoid bacillus; they are distinctly pathogenic for men and animals. In milk they produce no coagulation; they produce visible gas in glucose, but do not ferment lactose and sucrose; no indol is produced.* It is unnecessary to quote the amounts of the constituent gases produced in the fermentation of glucose. From a careful study of the bacillus "O" from his own case, and Gwyn's bacillus, Cushing believes that they could best be placed in a subdivision of the enteritidis group, as they are slower in their action in milk (alkali production) and grow less luxuriantly, resembling the typhoid bacillus in their growth in fluid media and as regards pathogenicity for animals.

According to Cushing, the colon bacillus is to be distinguished by its sluggish motility; its few flagella; its slight pathogenicity for man and animals; its abundant growth on potato; the absence of aërobic alkali production; its acidification and coagulation of milk; and the large amount of indol produced. Glucose and lactose are fermented with visible gas. The effect on sucrose varies.

The *B. typhosus* is to be distinguished by its active motility; its large number of flagella (up to fourteen); its marked pathogenicity for man and the lower animals; the slight acidification of milk, with little or no secondary alkalinity; the fermentation of glucose without visible gas; the absence of indol formation, or of any action on sucrose and lactose.

Durham¹⁷ in a later paper gives a very elaborate classification of the various organisms under discussion. Gwyn's bacillus and the bacillus "O" of Cushing are placed in a separate group (group C), being distinguished from the *B. enteritidis* group for the following reasons: According to him, these organisms produce visible gas only when the other constituents of the media are favorable; they grow more like the typhoid bacillus than do the members of the enteritidis group; they produce acid in litmus whey, whereas the members of the enteritidis group produce acid followed by

* As will be seen later, Cushing obtained an indol reaction with both his own and Gwyn's bacilli, and we have obtained slight reactions with nearly all of the paracolon bacilli we examined.

alkali formation. The sera of animals, immunized with the bacillus "O" and the bacillus of Gwyn, do not agglutinate the members of the enteritidis group.

I shall now give a brief summary of the organisms described in the cases of infection by members of the intermediate group, excluding those connected with the epidemics of meat poisoning and the cases of psittacosis.

All of the organisms described are typhoid — or colon-like — in morphology, are decolorized by the Gram procedure, and do not fluidify gelatin. The organisms isolated by Achard and Bensaude produced no indol, did not ferment lactose nor sucrose, did not coagulate milk, and were not agglutinated by typhoidal serum. The authors state that the organisms simulated the *B. psittacosis*¹⁸ very closely.

The organism isolated by Widal and Nobecourt is described as having properties like the bacillus isolated by Achard and Bensaude. These further data are given: It clouds bouillon without the production of a film; it produces visible gas in glucose; it ferments mannite, but not lactose nor sucrose; it grows as a yellowish-green film on potato and produces no indol; it does not coagulate milk. Widal and Nobecourt considered the organism to be closely allied to the *B. psittacosis*¹⁹ and the *B.* of calf septicemia of Thomassen.²⁰ Widal and Nobecourt's organism is evidently closely related to our own.

The organism of Malherbe and Mounier⁶ is simply described as producing no indol and not fermenting lactose — a description too brief to be of much service.

The organism of Gwyn, and Cushing's bacillus "O," resemble the bacillus from our case very closely. The only difference appears to be the later development of alkali in the milk cultures of their bacilli. The indol reaction was positive with their bacilli on the tenth day in sugar-free bouillon. On potato there was a slight yellowish growth after twenty-four hours; the potato was discolored after four days. On our potato-tubes the growth was the same as with our organism; *i.e.*, it varied on different potatoes show-

ing again that the latter medium is of value only within certain limits.

Schottmueller describes his organisms as being divisible into two groups — two in one and four in the other. Both groups are facultative anaerobes and produce gas in ordinary agar (media not sugar-free?). None of the bacilli coagulate milk, but after a few weeks the milk becomes translucent. In litmus whey the organisms in the first set produce a slight amount of acid; those in the second do the same, but the acid is followed by alkali production. Indol is not formed by either group. The first group gives but a slight growth on potato; the second a grayish-brown thick growth. No data are given as to their action on lactose and sucrose.

Through the kindness of Dr. Schottmueller and Dr. Norris I have been enabled to study one of each of the groups of Schottmueller's bacilli, the *Bacillus* "Mueller" and the *Bacillus* "Seeman." They both resemble our organism very closely. In litmus milk both produce acid followed by alkalinity; neither affects sucrose nor lactose. I obtained a slight indol reaction in sugar-free bouillon with the bacillus "Seeman" after eight days. (Schottmueller made his tests after four days.) On my potato-tubes both of his organisms gave a slight whitish growth. According to Schottmueller, all of his organisms produced a greenish-yellow fluorescence in neutral-red agar after twenty-four hours.

Kurth's organisms are not completely described. They have numerous flagella; produce visible gas in glucose; produce no indol (tested after two days). Milk was coagulated after several weeks. The organism retained its virulence for a long time. According to Kurth, it was to be distinguished from the *B. enteritidis* because boiled cultures were not toxic, and because sera that reacted positively with his organisms gave no result with the *B. enteritidis*.

The organism isolated by Buxton and Coleman, according to some notes kindly sent to me by Dr. Coleman, and the examination of a culture kindly furnished by Dr. Buxton, is very closely related to the organism isolated by Schottmueller, Cushing, Gwyn, and myself, but differs in that even after

six weeks there is no secondary alkali-production in litmus milk. Gas is formed in glucose and maltose. Lactose and sucrose are not affected. Litmus whey becomes acid and cloudy. A slight indol reaction was obtained by me in ten days in sugar-free bouillon.

In this connection it may be of interest to refer to an organism isolated by Guarnieri and Vincent²² in cases of acute yellow atrophy, at autopsy.* It was motile; grew less actively than the bacterium coli; produced indol; did not coagulate milk; fermented glucose rapidly, lactose and sucrose not at all; and acidified whey less than the bacterium coli. It was pathogenic for mice. Injected intraperitoneally into guinea-pigs it produced a septicemia with degeneration of the organs and marked changes in the liver. The organism was termed the *Bacillus icterogenes*. Similar organisms were found by Kruse and Pasquale²² in the stools in cases of typhoid fever.

Note: In my own work I did not make any use of the scraped-tube test²³ mainly because Theobald Smith²⁴ considered the test of no great importance. Cushing's results, however, are of some interest in that he found that members of the intermediate group would not grow well on a substratum of any of the allies.

From this summary it is evident:

First. That the bacilli isolated by Schottmueller, Cushing, Gwyn, and myself are practically identical in cultural characteristics. Buxton's bacillus differs only in its action on milk.

Second. That the organisms of Achard and Bensaude, Widal and Nobecourt, and Guarnieri and Vincent probably are identical.

Third. That these organisms are all easily to be differentiated from the typhoid bacillus and the colon bacillus.

How closely Kurth's organism may be related it is difficult to state. I have had no opportunity of studying it.

Note: Since writing the above Brion and Kayser have

* This organism, having been recovered post-mortem, is, of course, of doubtful significance from an etiological standpoint.

published a paper on a case of "paratyphoid infection" (Münchener med. Wochenschrift, April 15, 1902), in which they state that Kurth's bacilli closely resemble the bacillus "Seeman" of Schottmueller.

As regards the effects on animals, no extensive observations are reported regarding the members of this group. Achard states that three-fourths cubic centimeter of a bouillon culture of one of their paratyphoid bacilli killed a guinea-pig in less than twelve hours. No experiments were made with Gwyn's bacillus. Cushing states that the virulence of his bacillus "O" was like that of the *B. typhosus*. For fuller details I refer to his paper. I may state, however, that the serum of an animal immunized with his bacillus was possessed of no marked agglutinating power over other members of the intermediate group, but was possessed of a certain amount of protective immunity. Kurth's organisms were very virulent for guinea-pigs.

Quite a variety of names has been applied to the bacilli in the group under discussion. Achard and Bensaude called their organisms "paratyphoid bacilli." Schottmueller, without being cognizant of their work, used the same term. Widal and Nobecourt were opposed to this name because they believed that the organisms resembled the colon bacillus more than the typhoid bacillus. Kurth called his organism the "*bacillus gastricæ febricæ Bremensis*." We have stated above that Cushing classified his own and Gwyn's bacilli as a sub-group of the enteritidis group, and that Durham classified them separately.

As there is no definite arrangement as yet of the bacilli which possess some of the cultural characteristics of the colon bacillus and some of those of the typhoid bacillus, we believe that the choice of a name lies between the terms "paratyphoid" and "paracolon." The term "intermediate group" is apt to be misleading. Intermediate forms are apt to be found between other organisms.

There exists a number of organisms which are identical with the typhoid bacillus culturally, but differ from it in not being agglutinated by typhoidal serum. ^{7, 22, 47} We be-

lieve that the term "paratyphoid" if it be used at all should be applied to such organisms.

We have left, then, only the term "paracolon" for the group of bacilli under discussion. We appreciate that the name is not a very good one and that the organisms are probably as independent of the Bacterium Coli Commune as the latter is of the typhoid bacillus. Appropriate names for the organisms can hardly be forthcoming until the organisms in the typhoid-colon group can be properly classified.

Summary of Serum Reactions in the Reported Cases.

I have already referred to the reactions in the cases of Achard and Bensaude and to their lack of value. In the case of Widal and Nobecourt the reactions were as follows :

The serum of the patient agglutinated the paracolon bacillus in a dilution of one to one thousand. Colon bacilli were not affected. Another paracolon bacillus (their so-called "buccal" bacillus) was agglutinated in a dilution of one to one hundred and fifty; the *B. psittacosis* one to fifty; the Bacillus of calf septicemia was not affected at all. Serum from an immunized guinea-pig gave no reaction with colon bacilli, the typhoid bacillus, nor the buccal paracolon bacillus. Various normal sera gave no reaction. Immune sera produced with other bacilli (colon bacilli, bacillus psittacosis, and the buccal paracolon bacillus) were all negative with their paracolon bacillus excepting typhoid sera of very high agglutinative power. Fourteen typhoid sera all having an agglutinative power of one to one thousand or less with the typhoid bacillus did not affect their bacillus. An interesting experiment with one of the typhoid sera will be given later.

In Gwyn's case the serum reacted upon the isolated bacillus in a dilution of one to two hundred. There was no reaction with the *B. typhosus*. Two colon bacilli were agglutinated at one to fifty and one to sixty respectively, but normal sera affected them the same way. Typhoid sera did not affect the "Gwyn" bacillus.

In Cushing's case there was a positive reaction with the paracolon bacillus in a dilution of one to eight hundred after two hours. Typhoid serum and healthy sera gave no reaction. There was a reaction with an occasional variety of colon bacillus at one to fifty, but it was not marked. As mentioned above, he found no mutual serum reactions with the members of the intermediate group.

Schottmueller obtained reactions in very high dilutions in his case, but it is to be noted that he allowed three or four hours as the time limit of his reactions and did not regard the absolute cessation of motility as necessary for considering a reaction complete. The serum in his cases did not give any reaction with the *B. typhosus*, and typhoid serum gave no result with any of his organisms. The sera from his cases gave with their respective organisms reactions in dilutions varying from one to one hundred to one to ten thousand. The sera varied in their reactions with the bacilli recovered in the other cases. One serum reacted up to one to one hundred on all the organisms and on a colon bacillus. The second reacted on a number of the organisms and the colon bacillus. Upon the organism isolated from another case it reacted in a higher dilution than upon the organism found in the case. The serum of the third case reacted on the organisms from three other cases practically as well as on the organism isolated in the case. On two others it did not react at all and it gave a reaction in a dilution of one to one hundred with an ordinary colon bacillus. The serum from the fourth case reacted on the bacilli from two other cases in dilutions up to one to one thousand and one to ten thousand respectively, and in a dilution of one to fifty on two others. The fifth serum reacted markedly on three of the bacilli and only slightly upon two others. In the case of a physician who suffered from an attack of fever after having worked in the laboratory with one of the paracolon bacilli no organism was recovered, but a positive reaction was obtained with three of the organisms in a dilution of one to one hundred. The serum from a case of infection by the bacterium *coli* affected none of the paracolon bacilli, but only

homologous (that is, from the same case) and one heterologous colon bacillus.

In Kurth's cases, as noted above, he obtained two organisms and tested the sera of four cases. Positive reactions were found, varying in the respective cases from one to two hundred up to one to eight thousand. The *B. enteritidis* and the *B. typhosus* were practically not affected by these sera.

The serum in the case reported by Buxton and Coleman agglutinated their bacillus in high dilutions, but was practically negative as regards the typhoid bacillus, Cushing's bacillus, and Gwyn's bacillus. Experiments with immune sera of animals gave the same results.

With regard to our own observations we have already stated that we obtained no reactions with a number of the members of the intermediate group, whether we tested for an agglutination or for a thread reaction. The serum had already lost its power of affecting our own organism (the probable explanation of this will be given later), but still agglutinated the *B. typhosus*. We were unable to produce immune animal sera of high value on account of the accidental loss of our organism.

Summary.

It is very difficult to state exactly what we are to learn from all these results. The methods vary so much that it would hardly be proper to draw decisive conclusions. It can be stated in general terms, however, that the organisms in the various cases were agglutinated in high dilutions by the sera from the respective cases; that the organisms were not affected by typhoid sera, and the sera from the cases did not affect the typhoid bacillus. According to Schottmueller's data, even if we must accept them with some reserve, the serum from a given case may affect an organism from another case better than a homologous organism, and apparently similar paracolon bacilli are differently affected by the same serum. The fact that even with his liberal method of interpreting reactions some sera gave results in dilutions of

only one to one hundred, shows that in some of these cases very low reactions only can be expected. The reactions in our case show the same. It is also to be noted that a serum which gives a reaction in high dilutions with the bacillus isolated from a case may give absolutely no reaction with an organism apparently very closely allied. Thus Cushing obtained hardly any reaction when he tested the serum from his own case with Gwyn's bacillus in a dilution of one to ten, although he obtained a reaction in a dilution of one to eight hundred with his own bacillus. Similarly, Durham¹⁷ prepared a Gwyn serum which reacted in one to twenty thousand with Gwyn's bacillus, but has no effect on Cushing's organism in a dilution of one to one hundred.

It is evident that the paracolon bacilli resemble the colon bacilli in their peculiar behavior toward the sera of infected persons, — unless the various bacilli thus far found are to be regarded as separate species, — an assumption hardly warranted at present.

Summary of our Case.

The clinical picture of our case resembled that of a cholecystitis or liver abscess. The Widal reaction was negative for several days and then became positive in a dilution of one to two hundred and fifty. The paracolon bacillus was found in the gall-bladder, blood, and urine, and the blood gave a specific reaction with the various bacilli. Typhoid bacilli were not found at any time. At the autopsy, healed and healing ulcers were found in the ileum. It is natural to suspect that we are dealing with a case of typhoid fever (of the ambulatory type) in which a secondary paracolon infection occurred, and in which a Widal reaction appeared after the typhoid infection had practically run its course. In such a case the probable portal of entry would be either the typhoidal ulcers or the bile duct. The main objections to such an assumption would be the following:

First. That the presence of the Widal reaction depended upon the personal equation which is always present as regards this reaction.

Second. That the case was an instance of a positive Widal reaction in a person not suffering from a typhoid infection.

Third. That the patient had had typhoid fever at some earlier time.

Fourth. That the jaundice accounted for the positive reaction.

Fifth. That the positive Widal reaction depended in some way upon the paracolon infection.

Ad. 1. It is sufficient as regards this point to state that we never considered any reaction positive except when all motility had ceased, and all the bacilli were agglutinated.²⁸ In this particular case the reactions were all instantaneous.

Ad. 2. In an experience covering over one thousand cases of febrile diseases, we have never obtained a positive Widal reaction in cases that did not in one way or another prove to be instances of typhoid fever. In the cases reported in the literature in which reactions were obtained without the presence of a typhoid infection, the reactions were usually present in low dilutions, and were generally found by those who obtained very high percentages of positive reactions in the typhoid cases and obtained them early. As Cabot²⁸ states, practically all of these observations are doubtful.

Ad. 3. The reaction developed after it had been negative several days.

Ad. 4. This criticism would depend upon the statements of Koehler,²⁸ who says that the blood of a jaundiced patient may give a positive Widal reaction, independent of any typhoid infection. In his five cases, however, the reactions only reached one to forty at the highest. When it is noted that he used sugar bouillon cultures and considers clumps of four sufficient to term agglutinations, the results are of little significance. Our own experience with jaundiced patients speaks against the correctness of his views.

Ad. 5. The main data which might be cited in this connection all refer to the occurrence of a reaction, with organisms in the intermediate group (Gaertner's and Nocard's

bacilli) in supposed cases of typhoid fever.^{16, 27} These cases, if they are not instances of a mixed infection, would have to be explained by the work of Pfaundler on group-agglutination. This author found that in infections (particularly experimental ones) by a member of the colon group, a reaction was often present with other members of the colon group and the typhoid bacillus, but that the strength of the agglutination reaction with any of the latter organisms was less marked according to the degree to which the respective organism differed in cultural characteristics from the organism used for producing the infection. Widal and Sicard³⁰ have made similar observations.

The explanation of the positive Widal reaction in our case can hardly be made on the basis of its being a group-agglutination reaction, because the reaction against the typhoid bacillus was much more marked than the reaction against the paracolon bacillus. And the serum of one of the guinea-pigs inoculated with the paracolon bacillus gave a reaction against it in a dilution of one to fifty, but did not affect the *B. typhosus*. Furthermore, in none of the cases which I have cited in the introduction to this paper was there ever present a reaction with the typhoid bacillus. In the study of an epidemic of meat poisoning, made by Durham,²⁷ no reaction was found against the typhoid bacillus in any of the cases, although there were marked reactions with members of the Gaertner group of bacilli.

The occurrence of the lesions in the ileum in our case would also seem to point to the existence of a typhoid fever which had practically run its course before the patient came under observation. This statement cannot, however, be made with any degree of precision. As our own case is the first fatal one in the group, it is not known whether ulcers exist in the ileum or other parts of the intestine in these cases as they do in typhoid fever. Judging from some of the clinical histories (hemorrhage from the bowels), it is very possible. In other diseases caused by organisms of the intermediate group I am not aware of ulcers having been found. In psittacosis they have not been described.³¹

REMARKS ON MIXED INFECTIONS.

That there might be a secondary infection in typhoid fever by members of the colon group was seriously considered before the paracolon group was differentiated. Welch,³² in 1891, considered that typhoid ulceration might open the way to an invasion by colon bacilli. Guinon,³³ Chantemesse and Widal,³⁴ Péré,³⁵ and others, found colon bacilli in cases of cystitis and pyelonephritis in typhoid fever. Neisser³⁶ brought up the question of mixed infections in 1893. Keen⁴⁸ cites a case reported by Klemm, of a bone abscess due to a colon bacillus supposed to be secondary to an attack of typhoid fever, but the bacteriological examination is quite incomplete, judged by present standards. Achard and Bensaude suspected that their case might be one of mixed infection. Stern and Biberstein³⁷ thought that it was probable that there often existed a secondary infection by members of the colon group in typhoid fever cases because they found especially high agglutination reactions with the colon bacilli in some atypical cases. Johnson and McTaggart³⁸ from a study of serum reactions made the same suggestion. Cabot³⁹ states that Durham's observations suggest that what we call typhoid fever may be one of several infections, or a mixture of them.

In this connection the views of Meltzer,³⁹ recently published, are of interest. He reasons that because the reactions against the typhoid bacillus in cases of typhoid fever are so low compared with those obtained in infecting animals, and because the serum of typhoid cases also gives a reaction at times with one of the other organisms, there may exist mixed infections. He draws special attention to the possibility of mixed infections in which the typhoid bacillus is a secondary agent. In our own case, if it represents a mixed infection, as we are inclined to believe it does, the paracolon infection must have been secondary and the bacillus must have overgrown the typhoid bacillus. The abundance of the organism in the plates made from the blood made us feel positive that it would be impossible to find typhoid bacilli at the post-

mortem examination, even if they had been present in abundance before the secondary invasion occurred.

Cushing states that it is easy to understand the possibility, in fevers with intestinal lesions, of invasions by way of the portal circulation. But because it is unusual to find in lesions secondary to typhoid fever (excluding those occurring in the abdominal cavity) any organism except the *B. typhosus* or pyogenic cocci, he is inclined to believe that his case was not one of secondary invasion, but a pure paracolon infection. He claims to strengthen this argument by stating that if it were a case of mixed infection there should have been present a double agglutination reaction. But it must be observed that a number of months had elapsed since the supposed typhoid fever occurred, and the chances would be greatly in favor of a Widal reaction having disappeared in the interim even if it had been present. Gwyn, in his case, suspected the occurrence of a mixed infection.

Widal and Nobecourt cite an interesting observation. The serum from a typhoid convalescent agglutinated their paracolon bacillus at a dilution of one to twelve thousand, and the typhoid bacillus at one to twenty. Eight days later it agglutinated the paracolon bacillus at one to twelve thousand and gave no reaction with the *B. typhosus*. It agglutinated another paracolon bacillus at a dilution of one to twenty and an ordinary colon bacillus at one to two hundred. At the height of the disease there had been a reaction with the typhoid bacillus at a dilution of one to one hundred, and no reaction with the paracolon bacillus. Widal and Nobecourt considered the case one of typhoid fever with a secondary paracolon infection. These are all the observations there are on record as to the possibility of mixed infections with the paracolon bacilli. It will be noted that our case is the most suggestive one so far reported.

REMARKS ON THE THREAD REACTION.

The occurrence of a thread reaction in the course of the serum tests in our case is of some interest, and we believe it is necessary to make some reference here to the observations that

have been recorded in connection with this phenomenon in the study of members of the colon group.

The subject was first prominently brought forward by Pfaundler,⁴⁰ although other observers had previously noted the phenomenon. Before Pfaundler, Achard showed that the blood of animals (guinea-pigs) acquired agglutinating properties against colon bacilli with great difficulty only. Achard, and Widal and Sicard, demonstrated that the reaction against a colon bacillus which had caused an infection in the human body occurred only exceptionally. Widal had shown that it is difficult to say when an agglutination reaction with a given colon bacillus is pathologic. Pfaundler investigated eight cases of (non-systemic) infection by colon bacilli (cases of cystitis, peritonitis, etc.). In each of these he obtained a positive reaction with the serum of the case and the respective bacillus at a dilution of at least one to one hundred, if the hanging-drop preparations were observed after twenty-four hours. In the febrile cases he obtained the thread reaction; in the others, a clump reaction. The thread reaction presented the following appearance:

The organisms were arranged in long interwoven threads, the latter being generally coiled in skeins. The spaces between the skeins or threads were clear and none of the bacilli were motile. Very few single organisms or single groups of bacilli were found in the thread labyrinths. Upon observation with higher powers the organisms in the threads looked granular; some were club-shaped.* The bacteria could be demonstrated to be alive. The threads were best seen in the weakest dilution with which it was still possible to obtain a reaction. The threads usually appeared after a few hours. In most cases there was more or less agglutination at first. In Pfaundler's experiments he obtained positive reactions only when the patient's blood was tested with the organism causing the infection. He claimed, therefore, that the reaction represented more than a specific reaction, namely, an individualistic one. It is to be noted that the varieties of colon bacilli isolated from the cases varied very widely.

* In our own experiments we always found the threads appear like chains of streptococci.

In some unpublished experiments made by the writer in 1898 it was found that the thread reaction was frequently obtained in cases of appendicitis and peritonitis due to the colon bacillus. Only colon bacilli which were closely allied in cultural characteristics were used. Fever was present in all of the cases. The general results of the observations were that the thread reactions occurring in a dilution of one to twenty, if the reactions were complete, seemed to be specific, and no interaction between the various bacilli and a given serum was found. The experiments were too few in number, however, to prove distinctly the absence of any interaction. (In cases of proteus infection interactions were found.) In some cases agglutination reactions only were found.

Krauss and Loew⁴¹ obtained thread reactions with a number of organisms, with homologous and heterologous sera (that is to say, with the serum from the case from which the organism was isolated, and from other cases) and threw doubt on the claims of Pfaundler. However, their dilutions were in the main very low and the characteristics of the bacilli used in the experiments are not detailed.

Pfaundler⁴² in a second paper attempted to show by means of animal experiments that the thread reactions obtained with a given serum and bacillus occur only as an individualistic reaction. He states that the reaction is practically not as useful as the agglutination reaction, as it is inconstant.

Krauss⁴³ in a second paper came to the following conclusions:

First. That thread formation is a phenomenon which occurs with certain organisms under the influence of an agglutinating serum.

Second. Agglutination always precedes it.

Third. It is not as constant as agglutination.

Fourth. In general, the same rules that apply practically for agglutination reactions apply for thread reactions, except as regards the bacterium coli.

He bases his conclusions on the occurrence of thread-

reactions with homologous and heterologous coli sera. He obtained the reaction with normal serum also, and says that it is therefore not specific. He concludes, however, that threads and agglutinations usually occur in specific dilutions and with the use of a homologous serum and a homologous colon bacillus. It is to be noted, however, that a study of Krauss's experiments shows that most of his tests were made with low dilutions. Only once did he obtain a thread reaction at a dilution of one to fifty with a normal horse serum and a heterologous colon bacillus. It is questionable how much value such an experiment has as regards reactions in the human body. It was only with the use of a homologous serum and a homologous colon bacillus that he obtained the thread reaction in a high dilution (one to two hundred).

The reactions in our own case which have led up to this discussion were briefly as follows :

The organism obtained from the gall-bladder was affected at a dilution of one to one hundred on September fifth, but on September seventh, when the blood acted upon the bacillus isolated from the blood at a dilution of one to one hundred, the gall-bladder bacillus (which was identical with the blood bacillus) was affected at a dilution of one to twenty only and then not completely. On September eighth, the reaction with the gall-bladder bacillus was quite imperfect, and the reaction against the blood bacillus was present only at a dilution of one to fifty and not as perfectly as before. On September ninth, the results were about the same.

It was found that the sera of patients suffering from colon infections, which gave a good reaction with their own colon bacillus, did not affect our bacilli, nor did our serum affect colon bacilli from a number of cases of cholecystitis, appendicitis, and enteritis, and from the intestine of the patient. After September ninth, because of the pressure of work, we recorded reactions only at a dilution of one to twenty, and at this dilution we obtained thread reactions with all the bacilli isolated from the patient, for a number of days.

After several months the same serum which was preserved sterile, did not affect our organism. In this set of experiments agglutination did not precede the thread formation. All the organisms were involved in the reaction. Only occasionally did the threads persist for twenty-four hours; usually they were replaced by clumping, partial or complete, or the field was full of vibrating bacilli. The reactions were usually most pronounced after three or four hours.

The reactions obtained on September seventh, are particularly interesting, because the bacillus from the gall-bladder was much less affected than it had been the day before, whereas the bacillus obtained from the blood gave a reaction at as high a dilution as the bacillus isolated from the gall-bladder had given. Pfaundler observed that colon bacilli when grown outside of the body lose their power of being affected by the serum from the case, and adds this as an argument in favor of the individualistic character of the reaction.

We found, a few years ago, that the same serum and the same organism no longer gave a thread reaction after a number of days, if the organism was transplanted daily. The length of time varied for different organisms. It seemed to us also that the loss of the reaction seemed to be due to the artificial cultivation of the bacillus and not to a loss of power of the conserved serum. Further observations are needed on this point.

Flexner,⁴⁴ Kruse, and Duval and Vedder,⁴⁵ have noted the occurrence of the thread reaction with an organism more closely allied culturally to the typhoid bacillus than to the colon bacillus, namely, the dysentery bacillus. In their observations, however, it would appear that the thread reaction was of the same significance as the agglutination reaction.

It is important, we believe, to have this subject further investigated to ascertain to what extent agglutination reactions are to be depended upon for diagnostic purposes in cases of infection by colon and paracolon bacilli, and whether the thread reaction is of the same or of more value than the agglutination reaction.

Our case demonstrates that it is necessary in studying cases of infection by paracolon bacilli to make tests for thread reactions as well as for agglutination reactions.

According to our experiments, thread reactions are best obtained by making an emulsion in bouillon of a culture of the organism with agar. The suspension must be a very dilute one, just enough of the culture being used to cause a distinct cloudiness; usually two loopfuls of culture suspended in ten cubic centimeters of bouillon will suffice. Our dilutions were usually made with normal saline solution.

GENERAL REMARKS ON THE SERUM DIAGNOSIS OF PARACOLON INFECTIONS.

From all that has been stated regarding the reactions in the recorded cases, and the general remarks concerning serum reactions in the colon group, it is evident that it would be better for the present not to make an absolute diagnosis of a paracolon infection on the basis of the serum reactions alone. There seem to exist the same difficulties concerning the rules of agglutination as exist with the ordinary colon group.⁴⁶ It is important, at any rate, for the practitioner to know that there do not exist the same comparatively simple conditions as regards serum reactions as there do for the bacillus typhosus in cases of typhoid fever. A striking example of this is given by Durham,¹⁷ who found that two colon bacilli differing materially in cultural characteristics were agglutinated by the same serum, one at a dilution of one to fifty thousand, and the other at a dilution of one to thirty thousand. The serum had been obtained by immunizing an animal with the second bacillus.

If possible, the diagnosis of a paracolon infection should, for the present, rest on the recovery of a bacillus of the group, preferably from the blood or the urine.

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A COMPARATIVE STUDY OF THE BACILLI INTERMEDIATE
BETWEEN *B. COLI COMMUNIS* AND *B. TYPHOSUS*.

B. H. BUXTON.

In the fall of 1901 Dr. Warren Coleman and I undertook to make a series of cultures from the blood of typhoid patients in Bellevue Hospital.

Very early in our investigations we came across a bacillus from a supposed mild case of typhoid without Widal reaction, which proved to belong to the intermediate or paratyphoid group.

This led to the production of a joint paper read by Dr. Coleman before the New York Pathological Society on February 12, 1902, and subsequently published in the June number of the "American Journal of the Medical Sciences."

The main part of this paper was devoted to the clinical aspects of the paratyphoid infections, the bacteriological properties of the bacillus in question, here called Case 7, being also briefly touched upon.

Instigated by the possession of this bacillus, I collected a number of intermediates from various sources with the object of making a comparative study of them. My best thanks are due to those who so kindly supplied the cultures.

I am indebted for many of them to Drs. Cushing and Johnston, of the Johns Hopkins Hospital; to Dr. Schottmüller, of Hamburg, for two; to Drs. Libman and Hewlett, of New York, who each sent me a culture of the bacilli isolated by them. In addition to this a culture was received from the Hygienic Institute at Bremen, of one of the two bacilli isolated there by the late Dr. Kurth.

Into the details of the various cases it hardly seems worth while to enter. They have all been published with one or two exceptions, and references to them are given at the end of the article.

It can be gathered from the table that a large number of the cases were typhoidal in nature, the bacilli having been obtained for the most part from the blood.

Of the unpublished ones, Strong's case I understand was not typically typhoid, whilst Malcomb was a secondary infection after true typhoid, the bacilli having been isolated from the urine during convalescence.

The clinical picture of Libman's was that of cholecystitis complicating typhoid fever. The bacillus was obtained from the gall-bladder and twice from the blood during life, but since there was a positive Widal to typhoid at one to two hundred and fifty it was thought that there was probably a mixed infection. That the bacillus actually caused the typhoidal symptoms is therefore a little doubtful, but the doubt, having been here expressed, will not be referred to again, and it will be taken for granted that the bacillus was the cause of the typhoidal symptoms.

The following is a table of the organisms examined:

	From	Isolated by	Obtained from	Case.
1. Coli Communis.....	Buxton	Feces ...	Normal.
2. Hog Cholera	Moore Ithaca.			
3. Typhi Murium	Johns Hopkins.			
4. Gärtner B. Enteritidis	Johns Hopkins..	Gärtner, 1888	Feces ...	Meat poisoning.
5. Hatton	Johns Hopkins..	Durham	Feces ...	Meat poisoning.
6. Bac. O.....	Johns Hopkins..	Cushing	Abscess.	Typhoidal
7. Gwyn	Johns Hopkins..	Gwyn	Blood ..	Typhoidal.
8. Strong	Johns Hopkins..	Strong	Spleen ..	Typhoidal.
9. Malcomb	Johns Hopkins..	Johnston	Urine ...	Typhoid.
10. Badach	Johns Hopkins..	Johnston	Blood ..	Typhoidal.
11. Mllewsky	Johns Hopkins..	Johnston	Blood ..	Typhoidal.
12. Noonan	N.Y. Hosp.....	Hewlett	Blood ..	Typhoidal.
13. X	Mt. Sinai Hosp..	Libman	Blood ..	Typhoidal.
14. Müller.....	Hamburg	Schottmüller	Blood ..	Typhoidal.
15. Seemann	Hamburg	Schottmüller	Blood ..	Typhoidal.
16. K	Bremen.....	Kurth	Feces ...	Typhoidal.
17. Case 7.....	Bellevue Hosp. .	Coleman & Buxton.	Blood ..	Typhoidal.
18. Typhoid, 4 strains.				
19. Dysentery, 4 strains.				

A few remarks on some of the above may here be inserted. The culture of *B. coli communis* was selected as a typical one after comparison with several different strains. All these appeared to be precisely similar except that some of them fermented saccharose and others did not. Durham calls the former variety *Coli Communis Communi*or, since he considers it to be more frequently found in the feces than the latter, which he calls "*Coli Communis Verus*." The bacillus chosen here as a representative ferments saccharose, so belongs to the "*Communi*or" group.

Schottmüller during an epidemic of typhoid in Hamburg in 1900 isolated intermediate bacilli from the blood of six patients. Two of these, Müller (No. 14) and another, differed somewhat both culturally and in their agglutinating properties from the other four — Seemann (No. 15) and three others — and Schottmüller suggests that he may have been dealing, not with one, but with two nearly related groups of bacilli.

Kurth, during a typhoid epidemic in Bremen in 1900, isolated intermediates, one from the feces and the other from the urine in two cases which did not respond to the Widal test. In three other cases where the blood of the patients was negative to typhoid he obtained positive results with his intermediates. Which of these two was sent to me I do not know, but from Kurth's descriptions they appear to have been identical in every respect.

Four strains of typhoid were carefully examined and found to differ only in respect of the rapidity with which they were agglutinated. A stock laboratory culture originally obtained from the New York Health Board was selected for most of the comparative tests, since it agglutinated with typhoid serums more readily than any of the other three, all of which had been recently obtained from the blood of typhoid patients by Dr. Coleman and myself.

Four strains of the *B. dysenteriae* — those isolated by Shiga in Japan, Flexner in Manila, Kruse in Germany, Seward in New Haven — were also brought in for comparison, but these bacilli, although apparently identical, do not seem to

form part of the connecting links between *B. coli communis* and *B. typhosus*, and must be considered rather as side shoots of the group. According to published accounts, they resemble typhoid except that they are not motile and are not agglutinated by typhoid serums. They are not referred to in the article.

The *Bacillus fecalis alkaligenes* of Petruschky, from feces, appears also to be a side shoot from the group.

It is easy to distinguish the intermediates as a group from *B. coli communis* on the one hand and typhoid on the other.

The main points of difference are as follows :

	<i>B. coli communis.</i>	Intermediates.	<i>B. typhosus.</i>
Coagulation of milk	+	—	—
Production of indol	+	—	—
Ferm. of lactose with gas	+	—	—
Ferm. of glucose with gas	+	+	—
Agglutination by typhoid serum	—	—	+

When, however, the intermediates are more closely studied they are found to differ to a considerable extent among themselves, both culturally and in their agglutinating interactions.

As regards those which are pathogenic for man we find them clinically divided into three groups according to the symptoms produced.

(*a.*) The meat-poisoning group. (Gärtner, *B. enteritidis*.) Sudden onset soon after ingestion of the meat, symptoms at first those of toxin poisoning followed by invasion of the system with the bacilli. Duration of illness four or five days, after which quick recovery. In a few cases death in two or three days.

(*b.*) The pneumonic or psittacosis group.

This has been described by several French authors, Achard et Bensaude among others, and is ascribed by them to a bacillus first isolated by Nocard from a parrot supposed to have been the original cause of an epidemic. No cultures of this *B. psittacosis* could be obtained and it will not be

considered. Durham puts it with the Gärtner or Enteritidis group so far as its cultural characteristics are concerned.

(c.) Those causing typhoid-like symptoms.

We might then be inclined to divide the intermediates into :

I. The paracolons which do not cause typhoidal symptoms, including the meat-poisoning and psittacosis bacilli, together with those which are not pathogenic for man such as hog cholera, *B. typhi murium*, etc. ; and — II. The paratyphoid sub-group, to include all those bacilli which cause typhoid-like symptoms.

When, however, we come to consider the intermediates from the bacteriological standpoint we find that this division will not, without modification, hold good, for some of those which cause typhoid-like symptoms cannot be distinguished culturally from certain bacilli of Group I., whilst there are others which can be so distinguished.

The main object of this paper is to give in some detail the comparative experiments made, and to see if any satisfactory bacteriological classification can be arrived at. It must be understood that most of the tests, especially in cases where there seemed any prospects of their assisting in the classification, were made, not once or twice, but several times so as to eliminate, as far as possible, sources of error.

A few of the less important methods of differentiation may be touched upon to begin with.

Morphologically the intermediates cannot be distinguished among themselves, nor with any degree of certainty from *B. coli communis* or *B. typhosus*.

Biologically the differences on ordinary media such as agar, serum, gelatin, and bouillon are only those of degree and cannot afford much assistance for diagnosis.

Potato is notoriously an uncertain medium to deal with, and comparative trials were found to be misleading rather than helpful. As a rule those intermediates which are classified later on as Group I. grow less freely than *B. coli communis*, but the growth is distinctly visible, whilst Group II. resembles typhoid ; but these differences cannot be relied upon.

INDOL FORMATION.

Cushing found that his *Bacillus O* would produce traces of indol in nine or ten days when grown in sugar free broth, but on repeating the experiment he could detect no indol. Peckham observed that sugar free broth was superior to Dunham's peptone solution for experiments on the production of indol, so after a few negative trials with the latter sugar free broth was chosen for systematic comparative tests, which, however, were not carried on far, since it was obvious from the first that they would not assist in classification. One test was made in the following way:

Six tubes were inoculated each day for ten days in succession with *B. coli communis*, Gärtner, O, Gwyn, Case 7, and *B. typhosus*, and on the eleventh day all tested at the same time. It was found that *B. coli communis* began to show indol at the end of forty-eight hours; the intensity increasing day by day until a deep red color was produced. None of the others gave the reaction.

Two tests were made of the whole series with different lots of broth, one tube of each organism being grown for ten days. Malcomb gave reactions fully equal to those of the control *B. coli communis*, but none of the others showed more than a trace of color, not sufficient to register as positive.

Although a few authors claim to have observed slight indol formation by some of the intermediates, the majority have failed to confirm this, and it is evident that the indol test is unreliable at the best.

GROWTH ON SCRAPED-OFF CULTURES.

Widal and Nobécourt and some other French authors affirm that this is a useful method of distinguishing the intermediates as a group from the *B. coli communis* and *B. typhosus*. They tested it with a paracolon obtained from an abscess, Thomassen's bacillus of calf septicemia, and *B. psittacosis*. On growing cultures of these on agar for three days and then scraping off the growth they found that *B. typhosus* would not grow on the medium, but *B. coli com-*

munis grew quite freely. On the other hand, their three intermediates would grow on scraped-off typhoid cultures, but not on colon cultures, nor would they grow among themselves under these conditions. Achard and Bensaude obtained less positive results, but appear to think the method of value.

Cushing made some trials on these lines, but with very indifferent success, and my experiences coincide with his. Both serum and agar cultures were tried with very conflicting results which are not worth tabulating. Absolutely no assistance was rendered by them towards classifying the intermediates among themselves, and even as applied to the entire group the method appears to be of doubtful value.

SEMISOLID MEDIA.

Trials with five per cent gelatin and Hiss' semi-solid gelatine agar were made, both in culture tubes and by plating out, but with negative results.

Hiss' most recent medium for plating out was also tested.

Agar	15 grammes.
Meat extract . .	5 grammes.
Glucose	10 grammes.
Water	1000 cubic centimeters.

Although this medium is undoubtedly valuable for distinguishing between colon and typhoid, colonies of the intermediates usually resemble those of typhoid too closely to be distinguished from them with any certainty. No constant difference between the intermediates themselves could be observed.

Pathogenesis.

Every one who has at any time isolated an intermediate has tested its pathogenic properties and a review of all this work makes only one thing clear, and that is that it has advanced our knowledge — not one iota.

Experiments on the pathogenicity of the intermediates were therefore not attempted.

MILK.

Milk is not coagulated by any of the intermediates, but some of them, after initial acidity, produce by degrees sufficient alkali to dissolve the casein and so render the milk opalescent. This subsequent alkalinity does not occur with typhoid, the cultures becoming acid to litmus and remaining so permanently.

The alkali production occurs only in the presence of air, and Cushing, growing intermediates in fermentation tubes, found that the milk remained acid in the closed arm whilst the alkali production was confined to the bulb.

He noticed that his *Bacillus O* and Gwyn's paracolon bacillus produced much less alkali than the, at that time much better known, Gärtner group, and his photographs show that Gwyn's bacillus in four weeks appeared much less translucent than *Bacillus O*, although he does not mention this in the text.

Schottmüller lays great stress on the alkali production for the recognition of intermediates, but of his six cases, Barg and Müller only appeared slightly opalescent after several weeks, whilst the other four became decidedly so in two to four weeks. Durham has observed the same thing, but holds that the opalescence is of little value for diagnosis, since many other kinds of bacilli will cause a gradual clearing of milk.

However, granted that certain bacilli are intermediates, it is evident from Schottmüller's and Cushing's observations that there are differences among them in milk cultures, and it is worth while to consider if these differences will assist in grouping them. Several tests were made and the results found to be constant.

Coagulation in forty-eight hours, *B. coli communis*.

10 days. — Slightly opalescent. — Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seemann, Kurth.

20 days. — Distinctly opalescent. — Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seemann, Kurth. Average alkalinity five-tenths per cent phenolphthalein.

20 days. — Slightly opalescent. — *Bacillus O.* Neutral.

20 days. — Opaque. — Gwyn, Badach, Milewsky, Noonan, Müller, Case 7. Average acidity one per cent.

Control; acidity, one and five-tenths per cent.

The opalescence of the milk has always been attributed to the alkalinity, but the clearing is often slightly apparent even before the milk has reached the neutral point to phenolphthalein, so that it becomes a question whether it may not be due, partially at any rate, to production of casease by the bacilli.

Neither the intermediates nor typhoid will ferment lactose even to the extent of acid formation, but acidity, as first shown by Theobald Smith, cannot be produced except from a fermentable carbo-hydrate. Both typhoid and the intermediates can ferment glucose and other mono-saccharids, the former without and the latter with production of gas.

Typhoid produces CO_2 in glucose media, but not more than can be absorbed, so that there are no visible gas bubbles. In speaking of gas formation in this paper only visible gas formation will be indicated.

Theobald Smith, therefore, concluded that there must be a second sugar, probably glucose or galactose, present in milk, to the fermentation of which the permanent acidity of typhoid and the initial acidity of the intermediates are due.

It was thought, therefore, that if this second sugar could be previously got rid of the opalescence might appear earlier, and to effect this sterilized milk in bulk was inoculated with typhoid and incubated for three days. Then boiled, brought to its initial reaction, tubed, and after sterilization inoculated with the various organisms. The opalescence under these conditions was noticeable somewhat earlier, but there was no marked advantage in adopting this method.

LITMUS MILK.

Experiments with litmus milk are practically repetitions of those with plain milk, but the color enables one to judge at a glance if the culture is more or less alkaline than the controls.

Besides this, in cases where there is alkali production the litmus milk turns blue long before there is any visible opalescence in plain milk, so that there is a considerable saving in time in making a diagnosis.

It was found that after an initial slight acidity the milk would appear slightly more blue than the controls in about five days with Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seemann, Kurth, and the blue color would continue to increase in intensity; the average at the end of ten days being about five-tenths per cent to phenolphthalein.

Bacillus O is somewhat erratic. It will sometimes turn the milk blue almost as quickly as the above, and at other times will remain acid for a considerable time.

The following remain acid for ten days: Gwyn, Badach, Milewsky, Noonan, Müller, and Case 7 — the final acidity being about one per cent.

On previously treating litmus milk with typhoid there is only a trace of initial acidity with any of the intermediates.

In Two Days.

(a.) Slightly more blue than control: Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seemann, Kurth.

(b.) Like controls: *Bacillus O*.

(c.) Faintly acid: Gwyn, Badach, Milewsky, Noonan, Müller, Case 7.

In Ten Days.

(a.) Very blue: Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seeman, and Kurth. Average alkalinity, five-tenths per cent to phenolphthalein.

(b.) Somewhat less blue: *Bacillus O*. Neutral.

(c.) Like controls: Gwyn, Badach, Milewsky, Noonan, Muller, and Case 7. Average acidity one per cent.

Controls; acidity, one and five-tenths per cent.

The previous treatment with typhoid enables the differences to be observed much earlier, but there is no saving in time unless the medium is ready for use. It might, how-

ever, be found advantageous to prepare all one's stock litmus milk free of the second sugar in this way, so as to have it ready for an emergency. Alkali production could then be much more quickly noted at any time than with litmus milk not so treated, and this would no doubt hold good for many bacteria other than the intermediates.

Petruschky's Litmus Whey.

Durham places great reliance on this medium as a means of differentiating between the various colon groups including *B. lactis aerogenes* and *B. typhosus*, but it must be confessed that New York whey failed to afford the same results as he obtained with the English variety. He found that *B. coli communis* clouds the whey, and makes it permanently acid. The Gärtner group cloud it, but after an initial acidity there is in four days a production of alkalinity which goes on increasing, whilst with typhoid the whey remains clear but becomes permanently slightly acid. *Bacillus O* and *B. Gwyn* which he distinguishes from the Gärtner group behave in litmus whey very similarly to *B. typhosus*, in that there is no subsequent production of alkali and very slight cloudiness.

Schottmüller found ten days to elapse before there was sufficient alkalinity for the blue color to be regained, and as with milk his Barg and Müller differed from the other four; producing permanent acidity so that they were not distinguishable from typhoid. These observations could not be confirmed in their entirety. It is true that those intermediates which cause milk to become opalescent in ten to twenty days cloud the whey much more than the others. *Bacillus O* appearing to hold an intermediate position in this respect, but the acidity remained unchanged in all of them for three weeks, so that the only difference was in the degree of clouding, — a rather uncertain factor on which to depend for diagnosis.

If, however, the whey is first treated with typhoid as already described for milk in order to get rid of the second sugar, much more positive results can be obtained. In this case *B. coli communis* causes marked cloudiness and perma-

nent acidity, whilst typhoid cultures remain clear and form no acid.

There is initial acidity with all the intermediates, but in four or five days they begin to show differences :

- (a.) Cloudy and acid : Hog cholera and Seemann.
- (b.) Cloudy and slightly alkaline : Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, and Kurth.
- (c.) Slightly cloudy and alkaline : O.
- (d.) Slightly cloudy and acid : Gwyn, Badach, Milewsky, Noonan, Müller, and Case 7,

In three weeks.

Aver. alk.

Group (a) become alkaline in two to three weeks,	.1 per cent.
Group (b) become rapidly more alkaline	. 1 per cent.
Group (c) becomes more alkaline3 per cent.

Aver. acid.

Group (d) acidity permanent 2.2 per cent.
Controls5 per cent.

It is rather puzzling to account for the much greater initial acidity in litmus whey than in litmus milk when first treated with typhoid, but presumably typhoid is able to grow so sparingly in the former that it is unable to reduce all of the second sugar, which must here be somewhat more concentrated than in the original milk. In any case, however, so far as differentiating between the intermediates is concerned, litmus whey seems to be less valuable than litmus milk. Not only is it much more troublesome to prepare, but the alkali production is not noticeable so early as with the milk, even when it is previously treated with typhoid.

On reviewing the experiments with milk and whey we find that the intermediates fall into two distinct groups, Bacillus O appearing to hold a somewhat intermediate position.

The table then may be rearranged in accordance with the groups. Malcomb being put first since it forms indol freely and, therefore, appears somewhat nearer to B. coli communis than the others.

Group I.	Case.	Intermediate.	Case.	Group II.	Case.
1. Malcomb	Irregular.	10. Cushing's O.	Typhoidal.	11. Gwyn ...	Typhoidal.
2. Strong	"			12. Badach ..	"
3. Hog cholera ...				13. Milewsky.	"
4. Typhi murium .				14. Noonan ..	"
5. Gärtner	Meat poi- soning.			15. Müller ...	"
6. Hatton	Meat poi- soning.			16. Case 7....	"
7. Libman's X....	Typhoidal.				
8. Kurth	"				
9. Seemann	"				

GAS PRODUCTION.

It is generally recognized that the intermediates can be distinguished from typhoid by their power of fermenting the disaccharid maltose and all the monosaccharids with formation of visible gas. On the other hand they can be distinguished from *B. coli communis* by their inability to form acid or gas in lactose media, but Durham and Cushing, so far as I am aware, are the only ones who have published observations on differences in gas production among the intermediates themselves. Durham found that Gärtner and its allies would form gas from glucose and other monosaccharids in Dunham's peptone solution, but that *Bacillus O* and *B. Gwyn* would not. Dr. Hewlett, of the New York Hospital, informs me that he could not confirm this observation of Durham's, but finds that all the intermediates he has tested will form gas under these conditions.

Cushing remarked that *Bacillus O* and *B. Gwyn* in dextrose bouillon produced gas more slowly than the other intermediates — only about half as much in the first twenty-four hours — and the same was the case with mannite so far as *Bacillus O* was concerned.

In the following experiments it was found that all the intermediates would ferment maltose, glucose, galactose, fructose, mannite, dextrin, and yeast broth with production of gas, but not lactose.

Inulin, gum arabic, and calcium lactate were also tried with negative results.

Where peptone bouillon was used as a basis it was prepared sugar free by Theobald Smith's method, and after testing with *B. Coli Communis* one per cent of the sugar or other material was added.

Bent tubes of which the closed arm is one hundred millimeters in length were used, so that the measurements given in millimeters of the gas formed will afford an idea of the relative amounts.

The amount of carbon dioxid formed was tested in many instances by lime water, but this was not found to assist in the classification to any extent. As a general rule, however, there appeared to be a rather larger percentage in the cases where fermentation was rapid than where it was slow.

It was not thought worth while to test the percentage of acid or alkali formed. Occasional rough tests with litmus afforded no definite results, and it did not seem as if accurate observations would lead to anything.

GAS PRODUCTION.

SUGAR FREE BOUILLON.

Glucose, one per cent.

Fermentation complete in		Average gas in mm.		
		1st day.	2d day.	3d day.
1 day	<i>Coli Communis</i> , Strong	35-40	—	—
2 days	Malcomb, Hog cholera, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, Seemann	30	35	
	Highest first day, 39 mm. Lowest, 24 mm.			
3 days	O, Gwyn, Badach, Milewsky, Noonan, Müller, and Case 7	16	24	27
	Highest first day, 22 mm. Lowest, 10 mm.			
<i>Maltose, one per cent.</i>				
2 days	<i>Coli Communis</i>	21	30	—
	O, Gwyn, Badach, Milewsky, Noonan, Müller, Case 7	12	14	—
	Strong, Hog cholera	14	17	—
3 days	Malcomb, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, Seemann.	15	22	26

GAS PRODUCTION.

PEPTONE SOLUTION.*

Glucose, one per cent.

Fermentation complete in		Average gas in mm.		
		1st day.	2d day.	3d day.
2 days	Coli Communis.....	20	28	—
	Malcomb, Strong, Hog cholera, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, Seemann... }	15	25	—
	O, Gwyn, Badach, Milewski, Noonan, Müller, and Case 7	Bubble		
	<i>Maltese, one per cent.</i>			
2 days	Coli Communis.....	18	27	—
	Malcomb, Strong, Hog cholera, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, Seemann ... }	16	29	—
	Hatton, Malcomb, and Kurth formed a little more by third day.			
	Highest on second day, 33 mm. Lowest, 17 mm.			
	O, Gwyn, Badach, Milewski, Noonan, Müller, and Case 7..... }	8	11	—
	Highest on second day, 13 mm. Lowest, 9 mm.			

GAS PRODUCTION.

SUGAR FREE BOUILLON.

Galactose, one per cent.

Fermentation complete in		Average gas in mm.		
		1st day.	2d day.	3d day.
1 day	Coli Communis.....	37	—	—
2 days	Malcomb, Strong, Hog cholera, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, and Seemann... }	26	34	—
3 days	O,Gwyn, Badach, Milewski, Noonan, Müller, and Case 7	6	18	23
<i>Fructose, one per cent.</i>				
1 day	Coli Communis.....	40	—	—
2 days	Malcomb, Strong, Gärtner, Hog cholera, Typhi murium, Hatton, Kurth, and Seemann..... }	25	35	—
3 days	O,Gwyn, Badach, Milewski, Noonan, Müller, and Case 7	16	27	Trace more.

GAS PRODUCTION.

SUGAR FREE BOUILLON.

Mannite.

Fermentation complete in		Average gas in mm.		
		1st day.	2d day.	3d day.
2 days	Coli Communis	67	77	—
	Malcomb, Strong, Typhi murium, Gärtner, Hatton, Kurth, Seemann, }	38	48	—
3 days	Hog cholera, Libman's X	25	37	43
	First day, highest, 55 mm. Lowest, 24 mm.			
2 days	Badach, Müller	16	30	
3 days	O, Gwyn, Milewsky, Noonan, Case 7..	13	23	28
	First day, highest, 19 mm. Lowest 10 mm.			

The tables are compiled from two complete trials of the whole series in different batches of bouillon; other trials, each less complete in itself, afforded similar results.

Gas Production.

Yeast broth prepared by Spronck's method is uncertain. One batch failed to give any gas formation at all with any of the intermediates or *B. coli communis*, but as a rule there is a slight production of gas, more noticeable — about twelve millimeters — with Group II. than with Group I., — about eight millimeters, — and least of all with *B. coli communis*, which shows little more than a bubble.

B. coli communis and Group I. produce no further gas after the first twenty-four hours, but Group II. generally goes on fermenting for three days. But yeast broth is troublesome to prepare, and after the failure of one entire batch it was not tried again, since at the best it is inferior to glucose or mannite for differentiation. But the fact that *B. coli communis* in yeast broth forms less gas than any of the intermediates seems to indicate that the fermentable carbohydrate cannot be a sugar. Since dextrine would very likely be present in yeast, one per cent solutions in sugar free bouillon were tried with results very similar to those obtained by yeast broth; somewhat more gas, however, being formed by all the organisms. An experiment was made by growing *B. coli communis* in one per cent dextrine in bulk. After sterilizing and neutralizing it was put into fermentation tubes and inoculated with intermediates, but no gas was produced. It may be worth while to follow up this question of gas production in yeast broth and dextrin solutions at some future time, but it is doubtful if much can be learnt from the latter. The term dextrin, as proved by Brown and Morris, covers a multitude of inversion products intermediate between starch and maltose, and it is not likely that these would ever be present in the same proportions in the various commercial dextrins.

On reviewing the tables we find that there is distinctly less gas production by Group II., including *Bacillus O*, than by

Group I., and that the action by the former is usually somewhat slower.

The differences are best brought out by glucose, Group II. taking three days to complete the fermentation in bouillon, and producing only half as much gas as Group I., the highest quantity of gas with II. always falling below the lowest with I. Glucose in peptone solution is even better for differentiation, the mere bubble of gas formed by Group II. almost coinciding with Durham's observation that *Bacillus O* and *B. Gwyn* form no gas in this medium.

Photographs 3 and 4 show the progress of gas formation in glucose media, the marks indicating the gas formation day by day. Where the level of the fluid stands a little higher than the lowest mark this is due to contraction of the gas on cooling down after being taken out of the incubator.

Mannite also differentiates well, the highest of Group II. in twenty-four hours being five millimeters below the lowest of Group I.

With maltose, galactose, and fructose the fluctuations are greater, so that the differences between certain individuals of each group are not so great as the averages would seem to indicate.

COLORED MEDIA.

Rothberger in 1898 first advocated the use of neutral red agar for differentiation between *B. coli communis* and *B. typhosus*, the latter having no action on the color, whilst the former in twenty-four hours reduces it to a bright yellow with a marked greenish fluorescence. Since then neutral red agar has come into very general use, Makgill and Savage (1901) recommending it very strongly as a means of testing for the presence of *B. coli communis* in water.

Schottmüller tried it with his cultures and found that they reacted like *B. coli communis*, the color being changed to yellow in twenty-four to forty-eight hours.

The formula is:

Neutral plain agar	100 cubic centimeters.
Sat. aq. sol. of neutral red	1 cubic centimeter.
Glucose3 grams.

About eight to ten cubic centimeters are put into each tube and either deep stick or shake cultures made, the latter affording more uniform results. Very striking effects were obtained with this medium.

B. coli communis changes the color more rapidly than any of the intermediates, the yellow showing throughout the tube in twenty-four hours. With Group I. of the intermediates there is still, usually though not invariably, a narrow band of red at the surface in twenty-four hours, but in forty-eight hours the medium is entirely yellow. With Group II. there is no change in twenty-four hours except partially in the case of *Bacillus O*, but in forty-eight hours to three days the medium becomes yellow except for a depth of about two to five millimeters, at the surface. In both groups as with *B. coli communis* there is a considerable evolution of gas in twenty-four hours. *B. typhosus* and *B. dysenteriae* form no gas and the color remains permanently unchanged.

After four or five days the red color begins to return from above downwards with all the members of Group II., and gradually spreads until in three weeks or sometimes less the medium appears red again throughout, whilst with Group I. the yellow color is permanent. The only exceptions to this rule are Seemann and *Bacillus O*. About once in three or four trials the former would regain color though slowly, and the latter would not, so that these two appear to hold an intermediate position between the two groups so far as the reaction is concerned. None of the others ever failed to fall into their own group.

The colored plate serves as an illustration.

Neutral red, therefore, does not differentiate very clearly between *B. coli communis* and Group I., but is probably the most valuable medium we have for distinguishing the two groups among the intermediates themselves. No mention of this subsequent return of the color could be found in any of the published accounts, nor can any explanation of the reason for it be offered.

Toward sodium sulph-indiginate the intermediates react in the same manner, and the return of the blue-green color in

Group II. is even more rapid than with neutral red. But the color fades naturally even in the controls after eight to ten days, so that this dye will probably not prove to be reliable.

Other colored media were also tried and somewhat similar results were obtained with saffranin and methylene blue, but the reaction seems more uncertain. Fuchsin, night blue, Bismarck brown, and some other anilin dyes were also tried but with indifferent success.

Fermentation and colored media tests therefore show that the intermediates group themselves in the same manner as when tried with milk and whey, *B. Seemann* and *Bacillus O* appearing to be a little undecided as to which group they belong to. On the whole, however, *B. Seemann* appears to be more closely allied to Group I., and *Bacillus O* to Group II., so that they may be assigned to these respectively.

Culturally it is evident that there are two distinct groups among the intermediates, and of these Group I. appears, especially in regard to gas production and behavior in colored media, to stand nearer to *B. coli communis* than Group II. It is therefore proposed to call the groups paracolons and paratyphoids respectively, these terms appearing the more suitable in that, so far as is known at present, the members of Group II. cause no infections in man other than typhoidal.

It now remains to determine if these are groups in the sense that the various strains of *B. coli communis* form a group, or constitute two distinct species in the sense that the typhoid bacillus is a distinct species. Agglutination tests alone can help us to decide this question. Since the paratyphoids are so much alike in their pathogenic properties, it seems probable that they are a species rather than a group.

AGGLUTINATION.

A few of the published observations may be summarized as an introduction to the subject.

To begin with, Achard and Bensaude (1896), isolated two paratyphoid bacilli which were clumped by serum of the

patients, and slightly by typhoid serum. Three cultures of Nocard's *B. psittacosis* from different sources were clumped by the serum.

Widal and Nobécourt (1897) obtained a paracolon bacillus from a thyroid abscess, which was clumped (one to one thousand) by the patient's serum, but the serum was practically inactive (one to one hundred) on a whole series of allied forms, colons, paracolons, including *B. psittacosis*, and *B. typhosus*. Cushing (1900) found that serum of the patient from which his *Bacillus O* was isolated clumped it strongly up to one to eight thousand, but was negative to *B. Gwyn*, *B. coli communis*, and *B. typhosus*. Later the blood of a rabbit inoculated with *Bacillus O* clumped this bacillus and showed slight reaction to the bacillus of hog cholera. The latter was then injected, with the result that the rabbit's serum was active to *Bacillus O*, and the bacillus of hog cholera (one to five thousand), but practically negative to all others of the group including *B. Gwyn*.

Durham prepared a rabbit's serum with *B. Gwyn* which clumped it (one to twenty thousand), but had not the slightest effect upon *Bacillus O* (one to one hundred), whilst with serums prepared from the Gärtner group he found but few that would interact. He concluded, therefore, that the clumping test has only a limited value in the diagnosis of species.

Schottmüller observed that his *B. Seemann* and three others, which culturally belong to our Group I., would interact but slightly with Barg and Müller, Group II., but among themselves much more decidedly.

Kurth's two bacilli interacted, and were also clumped by the serum of three other typhoidal cases in which the Widal reaction was negative with typhoid.

It did not then appear on reviewing the literature as if the agglutination test would be of much value for classification purposes, but in this expectation I was agreeably disappointed, for a series of experiments on rabbits only served to accentuate the probability of Group II. constituting a distinct species and not merely a group.

A few preliminary remarks on the methods adopted may be inserted.

A number of rabbits were immunized to various organisms by subcutaneous injections of living agar cultures, beginning with one-twentieth or one-tenth and gradually increasing the amounts until a whole culture could be used without harmful results. Beyond this it was unnecessary to go, as the object was, not to get very highly potent serums, but to have them all as nearly as possible equally active towards their own organisms. The injections were made every week or ten days.

To take blood, the ear was shaved, dried, and the marginal vein pricked with a needle. A bulb pipette, as used for testing the alkalinity of the blood, containing five cubic centimeters and marked at twenty-five one-thousandths cubic centimeters on the stem, was found a convenient instrument; the blood being drawn up to twenty-five one-thousandths cubic centimeters and the bulb then filled with sterilized water.

This made the dilution one to two hundred and from this further dilutions could be made. The pipette was thoroughly washed out between each operation with distilled water, alcohol, and ether, but special aseptic precautions were not necessary, since the serum was always tested very shortly after being drawn.

It was found, however, that it could be kept perfectly well for two weeks in cold storage without losing its efficiency.

To test the reactions a number of small test tubes ($3'' \times 1/2''$) were taken and hung up side by side in a home-made wire rack. With a pipette one cubic centimeter of diluted blood was run into each of the tubes, and then one cubic centimeter of the culture to be tested; broth cultures not over twenty-four hours old being used for the purpose. The lowest dilution then for regular routine work was one to four hundred.

In looking over tables of reactions it is very confusing to find for example that one bacillus is acted on at one to ten thousand, another at one to two thousand, and a third at one to two hundred and so on. Such tables unless they are

closely studied are of no value in giving an idea of mutual reactions. It was thought therefore simpler and more practical to take a definite standard for interactions, and this was fixed, arbitrarily it must be confessed, at one to four hundred in four hours. It may be mentioned, however, that when there were mutual reactions at one to four hundred, these practically always occurred also at one to four thousand, in cases where the rabbit's serum agglutinated its own organism up to one to ten or twenty thousand.

Readings were taken in one, two, four, and twenty-four hours, and as soon as flakiness was visible to the eye this was marked as +. After the clumps had all fallen to the bottom of the tubes and the liquid above appeared perfectly clear, a ++ sign was used as an indicator.

(See Plate XVI. Fig. 4.)

Hanging drops were frequently made as controls, but where the action is closely watched it is not necessary to do this, except occasionally so as to make sure that one is not falling into error. It not infrequently happens, however, that the bacilli will gradually sink to the bottom in a cloud, without any previous visible flakiness, leaving the liquid above quite clear. If this process be watched it can be easily distinguished macroscopically from true reaction, but if the final stage alone be observed mistakes may easily arise unless a hanging drop be made, in which case only small atypical clumps can be seen, the majority of the bacilli being free and actively motile. It was found that typhoid more especially is liable to show this pseudo-reaction.

SERUM 1-400 IN 4 HOURS.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	
	Malcomb.	Strong.	Gärtner.	Kurth.	Seemann.	O.	Gwyn.	Case 7.	7 + Typhoid.	O. + X.	7 + Typhoid + Hatton.	
1. Coli	-	-	-	-	-	-	-	-	-	-	-	Coli.
2. Malcomb	+	-	+	+	+	+	-	+	+	+	+	Mal.
3. Strong	+	+	+	+	+	+	-	+	+	+	+	Str.
4. Hog Cholera ..	-	-	-	-	-	-	-	-	-	?	-	Hog.
5. Typhi Murium .	-	-	+	-	-	-	-	-	-	-	-	Mur.
6. Gärtner	-	-	-	-	-	-	-	-	-	-	+	Gärt.
7. Hatton	-	-	-	+	+	-	-	-	-	+	+	Hatt.
8. Libman's X ...	-	-	-	+	+	-	-	-	-	+	-	X.
9. Seemann	-	-	-	+	+	-	-	-	-	+	-	Seem.
10. Kurth	-	-	-	+	+	-	-	-	-	+	-	K.
11. O	-	-	-	-	-	+	+	+	+	+	+	O.
12. Gwyn	-	-	-	-	-	+	+	+	+	+	+	Gwyn.
13. Badach	-	-	-	-	-	+	+	+	+	+	+	Bad.
14. Milewsky	-	-	-	-	-	+	+	+	+	+	+	Mil.
15. Noonan	-	-	-	-	-	+	+	+	+	+	+	Noon.
16. Müller	-	-	-	-	-	+	+	+	+	+	+	Müll.
17. Case 7	-	-	-	-	-	+	+	+	+	+	+	7.
18. Typhoid	-	-	-	-	-	-	-	-	-	+	+	Typh.

Group I.

Group II.

The accompanying table is given as an example of the results obtained. The Gwyn rabbit unfortunately died before its serum was tested with all of the cultures. Eleven serums were used in all, over five hundred tests being made at the standard dilution besides a large number of others. No. 9, called 7 + Typhoid, was prepared by injections of equal quantities of Case 7 and typhoid, and was found to agglutinate all of Group II. and typhoid. Later on these were discontinued and Hatton used for injections. The serum No. 11 then agglutinated Hatton in addition to the others. After the Bacillus O serum had been thoroughly tested, injections of Bacillus O were left off and X used instead. The serum No. 10 then agglutinated X, Kurth, and Seemann without losing its activity towards Group II. The results obtained were remarkably constant, the only exception being that the Bacillus O rabbit after inoculation with Bacillus O for two months and then one month with X was found to agglutinate the Bacillus of hog cholera, though slowly and somewhat imperfectly.

Cushing, as has already been remarked, observed that the Bacillus of hog cholera reacted slightly with the serum of his Bacillus O rabbit. Although it has not been mentioned before, it was found in the course of these experiments that the Bacillus of Hog cholera has a tendency to form rather less gas and alkali than other members of Group I., so that it may have some affinities with Group II. Still the evidences in favor of this are so slight that they will not be taken into consideration in classifying.

It will be noticed from the table that B. Strong reacted readily with all of the serums.

This led to its being tested with normal rabbit's serum, by which it was agglutinated just as quickly, even up to one to four thousand. It was also found to be agglutinated one to four hundred by normal serum of man, and guinea-pig; but not by that of a monkey or dog. No explanation can be given for this extraordinary fact.

Nor can any explanation be offered for the failure by Cushing and Durham to get interactions between Bacillus O

and B. Gwyn. There was no question about it at all in my experiments, and, in doubt at one time as to whether mine was really a *Bacillus O* culture, I wrote to Dr. Johnston of the Johns Hopkins laboratory, who kindly sent me another, which, however, behaved culturally and in its agglutinating properties precisely like the first.

Dr. Johnston writes that in his own experience *Bacillus O* interacts imperfectly with other members of Group II. Dr. Hewlett, however, finds that *Bacillus O* is agglutinated by B. Noonan serum.

On examining the table we find that all the members of the paratyphoid group interact freely, quite as readily in fact as various strains of typhoid would be likely to do. It appears therefore that we must here be dealing with a distinct species, the members of which are indistinguishable from each other either culturally or by agglutination tests, and can cause a typhoid-like infection in man.

Turning to the paracolons, however, we find that the two which are not pathogenic for man, the bacillus of hog cholera and *B. typhi murium*, do not react with any of the serums prepared, with the doubtful exception already mentioned, nor do the serums of Strong and Malcomb take effect on any but their own organisms. Neither of these, however, caused meat poisoning or typhoidal symptoms in man.

The meat-poisoning group, of which only two, *B. Gärtner* and *Hatton*, were tested, is a very large one and the number of bacilli known to belong to it has been constantly increasing since Gärtner first isolated his in 1888. It is not worth while to refer individually to the numerous published accounts of cases where the bacilli have been isolated, but they all tend to show that the members of the group are culturally similar, and belong to our Paracolon group. But when tested for their agglutinating properties it becomes evident that there are differences between them.

The observations of Durham and Fischer may be briefly summarised.

The former found that the races Gärtner and Moorseele were strongly affected by Gärtner serum, whilst the races

Hatton, Basenau, Gand, Günther, Sheffield,* and some others were little or not at all affected.

Bernard Fischer, who isolated bacilli from meat-poisoning cases in the towns of Haustädt and Rumpfeth, near Kiel, observed that they showed mutual reactions and interacted also with Gärtner and Van Ermenghem, slightly with Kaensche and Günther, but not at all with Basenau, Grünthal or Glückstadt.*

On consulting the accompanying tables it will be seen that *B. Gärtner* and Hatton did not interact.

Enough then has been done to show clearly that taken all together the bacilli causing "meat-poisoning" epidemics are not a distinct species within the paracolons group, but simply members of that group; a group made up of various races just as is the case with the *B. coli communis* group.

From among the paracolons tabulated here, however, we can pick out three which do show mutual reactions and, unlike the "meat poisoners," appear to constitute a distinct species within the group: namely, Libman's X, Kurth, and Seemann, and it is precisely these and these only which were isolated from cases resembling typhoid fever, Malcomb and Strong having been ruled out.

It is interesting to observe that they came from New York, Bremen, and Hamburg respectively.

It would be necessary to compare a larger number of these before any definite conclusion can be reached as to whether they constitute a distinct species or not, but they may provisionally be put in a separate sub-group.

According to their cultural properties they should belong to the paracolons, but since they cause typhoidal symptoms, and are here assumed to be a distinct species, it seems better to class them with the paratyphoids, distinguishing between them and the first mentioned species.

The following classification is therefore suggested:

Paracolons.—Those which do not cause typhoidal symptoms in man. A group containing numerous different members, but culturally alike.

* These names represent various strains of the meat-poisoning group.

Paratyphoids.— Those which cause typhoidal symptoms.

a A distinct species culturally unlike the paracolons.

β A distinct species culturally resembling the paracolons.

The question then arises whether in cases of suspected typhoid which are negative to Widal with typhoid cultures it is likely to prove advantageous to try the serum with other organisms.

There seems little doubt that a test with an *a* paratyphoid may in some cases be of assistance in diagnosis, and it is quite possible that the *β* paratyphoids may prove useful in some instances. If the above classification is found on further investigation to hold good, it will certainly simplify matters for the clinical pathologist, since he will be able to make tests with intermediates in a systematic manner instead of blindly proceeding with any culture he may have in stock in cases where his typhoid cultures fail him.

Since finishing the above Dr. Longcope of Philadelphia has kindly sent me, with permission to mention them, cultures of intermediates obtained by him from the blood of two patients with typhoidal symptoms at the Pennsylvania Hospital. One of these, Case 4799, belongs clearly, both in its cultural and agglutinating properties, to the *a* paratyphoids, whilst the other, Case 5207, culturally resembles the paracolons and *β* paratyphoids, but is not agglutinated by any of the serums.

All further work done on the *a* paratyphoids has only helped to confirm the opinion that they are a distinct species, but on immunizing more highly the *β* paratyphoid rabbits, Seemann serum has developed the power of agglutinating the bacillus of hog cholera, *B. typhi murium*, and *B. Hatton*, though somewhat imperfectly. Kurth and O + X, however, continue to act as already described. This observation and the fact that Dr. Longcope's 5207 is not affected by any of the serums tend to render doubtful the likelihood of our being able to recognize a *β* paratyphoid species; but I shall hope to return to this work later on and endeavor to

answer some of the unsolved problems afforded by the alkali producers.

Strong has just published his case in the May number of the "Johns Hopkins Bulletin." It appears to have resembled typhoid clinically, but the author leaves it an open question as to whether the bacillus isolated from the spleen after death was actually the cause of the infection or not.

NOTE. — In the *Münchener Medizinische Wochenschrift* for April 15, 1902, has appeared a description by Brion and Kayser of Strassburg of an intermediate bacillus isolated from the blood of a patient with typhoidal symptoms in October, 1901. The serum of the patient clumps this bacillus, and also Schottmüller's Müller, but not Seemann, whilst the bacillus itself is not an alkali producer, and forms gas more slowly than *B. coli communis*. It appears then to be an *a* paratyphoid, and this, including Schottmüller's Barg, brings the number of those described within the last three years up to ten, eight of which have been under observation here.

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DESCRIPTION OF PLATE XV.

FIG. 1. Cultures in milk tubes. Three weeks old. Left, Group II. Center, Cushing's Bacillus O. Right, Group I.

FIG. 2. One per cent glucose in sugar free bouillon. Left, *B. coli communis*. Center, Group I. Right, Group II.

PLATE XVI.

FIG. 3. One per cent glucose in peptone solution. Left, *B. coli communis*. Center, Group I. Right, Group II.

FIG. 4. Agglutination tubes. Left, no agglutination. Center, some agglutination. Right, marked agglutination.

CHEMICAL NATURE OF THE BILE AFTER CHOLECYSTOTOMY.

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The cause of the precipitation of the biliary ingredients was first sought by me in efforts to produce gall stones by artificial means. Various efforts were made with different solutions of ox bile, lime water, mucin, cholesterin, and portions of fibrin. Then mechanical means were added, such as constant dropping of bile surcharged with lime salts upon fibrin at brood oven temperature. All these efforts were futile, but the curious fact was noted that when fibrin was allowed to remain in bile the bilirubin was deposited upon the fibrin, and thereby the fibrin was protected from decomposition by the bacteria, while fibrin placed in water under similar conditions soon becomes dissolved by the action of the enzymes of mixed bacteria by infection from the air. In spite of the excellent work of Richardson and others in demonstrating the agency of typhoid bacilli in the production of gall stones it seemed that there must be some chemical change in the nature of the bile itself to produce the results. After some waiting an excellent opportunity was offered to obtain bile directly from a fistulous opening, immediately after cholecystotomy performed for the removal of gall stones. Unfortunately I was not able to obtain samples of these stones for analysis, owing to inadvertence. For one of these opportunities I am indebted to Dr. E. W. Cushing, and for the other to Dr. S. J. Mixter, through Dr. Donoghue. In both cases the common duct was completely occluded. In the first case, during the forty-eight hours subsequent to the operation nine hundred and two cubic centimeters was obtained, and on the third day four hundred and two cubic centimeters. In the second case the amount for the three days on which it was collected was respectively three hundred and seventy-eight and eight-tenths grammes

first day; three hundred and ninety-six and four-tenths grammes second day; four hundred and twenty-five and six-tenths grammes third day. All of the specimens were of a reddish-brown color, rather viscid, and possessed a slight musty odor. The peculiarities of pathological bile, if it may be so called, are not very well known except from experimental sources. Hoppe-Seyler has found the bile in the gall bladder entirely free from cholates, and from this he argues that one source of the cholesterin stones which are so commonly found in the gall bladder is the disproportion between the cholesterin and the cholalic acid, which as a cholate is the natural solvent of the former. Again, in the gall bladder when opened, there has been frequently found a mass of slimy substance which may be either mucin or nucleo-albumen. By closure of the common duct experimentally in dogs Dochman found that the calcium increased and the sodium diminished proportionally to the time that the flow of bile was obstructed. Furthermore, Harley has found that under similar conditions the taurocholic acid was very much diminished, as compared with the normal, while the cholesterin was increased. In the two examples which are the base of these investigations it is evident that with complete closure of the common duct in both individuals, a closure so complete that drainage through the abdominal wall was necessary, the conditions were somewhat similar to those produced experimentally by Harley and others, while at the same time it is inconceivable that the return of normal conditions would have occurred at once, so that we are to conclude that if abnormal conditions could be found in the bile they had something to do with the deposition of the stones.

In the investigations made, special attention was given to the elucidation of three points:

First. The amount and peculiarities of the mucin found.

Second. The ratio of cholalic acid to cholesterin.

Third. The ratio of sodium to calcium.

Incidentally investigations into the alkalinity of the bile were made, as well as the relative amounts of taurin and glycocoll.

METHODS.

Three different methods were employed for the isolation of these different products, and a short critical résumé of these may not be without value. The precipitation of mucin can be accomplished by two different ways, viz., by alcohol or by acetic acid, and the precipitate thus produced can be largely washed free from the bile pigments adherent by alcohol or ether. This method gives a fairly pure product which can be easily dried and weighed. If it is desired to redissolve and reprecipitate the precipitated mass, this must not remain long under alcohol, else the mucin becomes coagulated and resolution is impossible. If acetic acid be employed it is very necessary to redissolve and reprecipitate several times (three times, at least, this was done in my work) in order to obtain a product free from bile pigments. If it is not desired to obtain quantitatively the amount of mucin, or if another portion of bile be used for other constituents, the addition of animal charcoal facilitates very much the removal of the pigments, but it is not a perfectly safe procedure, since the cholates cling closely to the charcoal, and are removed with difficulty. After evaporation to dryness, the repeated extraction of the residue with hot absolute alcohol affords a solution in which the cholates can be precipitated by a large volume of ether, but without the use of charcoal this first precipitate is highly colored with pigments and requires a second precipitation. When this is done the solution of the first precipitate is an incomplete one, a fact to which Hammarsten has already called attention, and this insoluble residue was collected and destroyed for estimation of sulphur, nitrogen, and phosphorus. There was only a trace of phosphorus, showing the absence of lecithin, but the residue from five hundred cubic centimeters of bile, obtained in this way, contained .0045 grammes sulphur when reckoned from the BaSO_4 found, and another five hundred cubic centimeters contained .0112 grammes nitrogen, or a ratio of nitrogen to sulphur of 112 to 45.

The ratio of N. to S. in taurocholate of sodium is 46:100, so that this residue must be made up largely of the glycolate

of sodium, since the nitrogen so largely predominates. As my object was to obtain from these salts cholalic acid for estimation, attempts were made to separate the taurin and glycocoll by the addition of strong hydrochloric acid to their watery solution, but in vain; while strong hydrochloric acid will separate the glycocoll as a chloride and the taurin as free in concentrated solutions of the pure salts as I was able to demonstrate, it was entirely ineffectual in these dilutions, containing, as they do, some impurities obtained by the above process; hence it was necessary to cook these cholates in a closed flask to which a cooler was attached with twenty-five per cent of the volume of the solution of hydrochloric acid, 1.12, for several hours, then the filtered, washed, precipitate an equal time with weak NaOH solution, acidify, evaporate, and extract with absolute alcohol, by which the free cholalic acid was obtained. To separate taurin from glycocoll chloride Hammarsten recommends that they both be dissolved in five per cent hydrochloric acid solution and then taurin be separated by adding ten volumes of alcohol. In my hands, possibly from lack of experience with the method, it proved utterly futile; at first the solution was difficult and then the addition of alcohol did not cause the taurin to separate, even after six months' standing, to the slightest degree, and recourse was had to the older method of dissolving out the glycocoll chloride with alcohol, leaving the taurin. The ethereal filtrate, after the removal of the cholates by filtration, was found the most convenient for the estimation of cholesterin, which was easily done by evaporating off the ether, leaving an alcoholic solution to which KOH was added and the whole heated until complete saponification, the alcohol driven off, the residue redissolved in water, and the cholesterin shaken with ether. For the sodium and calcium, incineration of the residue always took place with the slightest perceptible glow of the crucible, the charred mass cooked out with hot water, filtered upon an ash free filter, well washed with hot water, and the filter and contents dried and heated to a red heat in the same crucible until a white ash appeared. This was first extracted with hot water

which was added to the previous watery extract and the whole used for the determination of sodium and potash, while a subsequent extraction of the same ash with dilute hydrochloric acid served for the determination of the calcium. Incidentally the phosphorus in the alkaline solution from which the cholesterin was removed by ether was made, and since this phosphorus could only come from lecithin, this gave a measure of that substance in the bile.

RESULTS.

Water. — In the first two samples of the first bile examined, one a mixture of the first two days' discharge and the other that of the second day, a portion was weighed out, evaporated, dried at 105–110° C., and the residue weighed. In the second bile ten cubic centimeters were measured out with a pipette, dried, and weighed. The result was that 14.7318 grammes bile contained .2252 grammes residue, or 1.526 per cent, and 10.6894 grammes bile contained .1497 grammes, or 1.419 per cent. Of the second bile ten cubic centimeters contained .10689 grammes residue, or 1.068 per cent. This gives the water in the bile respectively, 98.47 per cent, 98.58 per cent, and 98.93 per cent.

Mucin. — This, as has been mentioned, was twice precipitated by alcohol, and once by acetic acid, and the latter three times redissolved and reprecipitated. First sample (bile No. 1), two hundred cubic centimeters gave .32249 mucin, after being precipitated three times with acetic acid, .166 per cent, or 10.8 per cent of the residue. The nitrogen of this mucin was determined by Kjeldahl and found to amount to 12.1 per cent, which demonstrates that this was almost a pure mucin, since the nitrogen of mucin is given by Neumeister as from 11.7 per cent to 12.3 per cent. The second one became coagulated by the alcohol, by remaining too long under it, and could not be redissolved by NaOH; as it was mixed with bile pigments, no account was taken of it. In the second case fifty cubic centimeters contained .0664 grammes mucin coagulated by alcohol, .133 per cent based on the bile, or 12.6 per cent of the residue. This

gave .0105 grammes of nitrogen, or 15.8 per cent; from this one would judge that in this bile the alcoholic precipitate was more nearly of the nature of a nucleo-albumen, or had not been washed free from bile coloring matters.

ALKALINITY.

Every sample of bile was alkaline to litmus and to phenolphthalein. In the second sample of No. 1 an effort was made to determine quantitatively the degree of alkalinity by the method used for blood. Five watch crystals were prepared, each containing respectively, .05, .1, .15, .2, .25 cubic centimeters $\frac{1}{2}$ normal tartaric acid, and to each .1 cubic centimeter bile added; then after stirring, a drop from each was placed upon a drop of alcoholic solution of phenolphthalein on pipe clay. A stage of neutral reaction was reached between .1 and .15 cubic centimeters N-25 tartaric acid, or an alkalinity equivalent to two hundred milligrammes NaOH to one hundred cubic centimeters bile.

CHOLALIC ACID.

This was obtained in the manner mentioned above and purified by at least two extractions with alcohol, at first of the residue produced by evaporation of the acidified cholates, and after the subsequent precipitations with ether, an extraction of the dried precipitates by ether, which latter precipitate was dried and weighed. The polariscopic reading was also taken of the precipitate after weighing, in alcoholic solution. Of the one bile the cholates were first precipitated and then split by hydrochloric acid, while in the second one the cholalic acid was obtained directly from the bile by cooking. Their purity was always determined by microscopic examination. And usually after the second precipitation with ether large octahedral colorless crystals, unmixed with amorphous matter, were found. Of bile No. 1, one hundred and fifty cubic centimeters of the first specimen gave .0092 grammes of cholalic acid, or .0061 per cent; one hundred cubic centimeters of the second gave .0092 grammes cholalic acid also, or .0092 per cent. Of the second bile,

one hundred and fifty cubic centimeters gave .0072 grammes, or .0048 per cent.

CHOLESTERIN.

The ethereal solution of the cholesterin after saponification was washed by shaking with water, freed from ether, redissolved in a small amount of hot alcohol, and allowed to crystallize by cooling. In this way microscopically pure crystals of cholesterin were obtained, which were dried and weighed. Two hundred cubic centimeters of the first sample of bile No. 1 gave .05569 of this substance, or .0278 per cent, while two hundred and fifty cubic centimeters of the second sample gave .06089, or .0243 per cent; fifty cubic centimeters of the second bile gave .01669, or .0334 per cent. By a comparison with the previous figures, it will be seen that the ratio of cholalic acid to cholesterin in the first sample of No. 1 is .01227 to .0556, or 1:4.53; in the second sample of the same the ratio is .023 to .0608, or 1:2.9. In the second bile .0024 to .0166, or 1:6.9.

CALCIUM AND SODIUM.

It was found that practically no calcium in the ash was soluble in water and that there were only traces of potash, practically all of the calcium existing as in water insoluble neutral phosphate and all the chlorine as sodium chloride. From the solution of the chlorides, after weighing, the potash was precipitated by plat. chloride and alcohol, filtered, dried, weighed, and KCl, estimated from plat. potash chloride, deducted from the weight of the chlorides. The Na was calculated from the chloride and calcium from the oxide. In the first specimen of No. 1, 14.73189 contained .0401 gramme Na and .00057 gramme Ca, in the first estimation, or a ratio of 70.4 to 1; in the second, two hundred cubic centimeters contained .52595 gramme Na and .00599 gramme Ca, or a ratio of 87.8 to 1. In the second sample of No. 1, two hundred and fifty cubic centimeters contained .4552 grammes Na and .00385 grammes Ca, or a ratio of 118 to 1.

Of the second bile, ten cubic centimeters gave .05749 Na and .000649 Ca, or a ratio of 88.6 to 1.

LECITHIN.

The residue, after saponification and removal of the cholesterol with ether, was cooked with $\text{Ba}(\text{OH})_2$, cooled and filtered, the excess of barium removed by CO_2 , filtered, evaporated almost to dryness, and this residue destroyed by H_2SO_4 and Amm. nitrate, and the phosphorus determined as pyrophosphate of magnesium. As this residue was soluble in alcohol and formed a soluble salt with barium, it must have come from lecithin and can be used as a means of estimating that substance. Two hundred cubic centimeters of the first sample gave .02029 grammes pyrophosphate, or .00569 phosphorus, and as the average phosphorus in lecithin amounts to 3.94 per cent, two hundred cubic centimeters bile must have contained .142 grammes lecithin. Two hundred and fifty cubic centimeters of the second sample contained .0264 grammes pyrophosphate of magnesium, obtained in the same way, or .00727 grammes phosphorus, or .184 grammes lecithin. In the second bile only traces of lecithin were found, too small for estimation. In order to compare these results better with each other, and also with results already published, particularly with those of Jacobsen, Pfaff, Balch, and Hammarsten, I append a table in which the constituents are expressed in parts per thousand in order to avoid long fractions.

FIRST BILE.	Solids.	Mucin.	Water.	Chol. acid.	Choles.	Lect.	Na.	Ca.
Sample a.	15.28	1.66	984.7	.061	.278	.710	2.625	.0299
Sample b.	14.19		985.8	.092	.243	.736	1.820	.0154
Second bile.	10.68	1.32	989.3	.048	.323	trace	5.74	.064
Jacobsen's case.	22.6	2.3	977.4		.560	.050	3.121	.088
Hammarsten's cases.	25.2	5.29	974.8	7.614	.630	.574		
Bile from fistula	25.4	5.15	974.6		1.5	.650		

Sodium calculated from the carbonate, chloride, and phosphate, and cholalic acid from the glycocholate and taurocholate of sodium. The fistula in Jacobsen's case had persisted for months.

CONCLUSIONS.

Upon examination of the figures before us, we note a somewhat diminished amount of solids as compared with Jacobsen's and Hammarsten's results. It is true that their investigations were made upon individuals whose fistulas had existed much longer than in these patients and the liver probably had regained in a large measure from its temporary functional incapacity. We can easily picture to ourselves that the liver cells, though not pathologically changed, from obstruction to the flow of bile pass through a state of temporary insufficiency from which they as rapidly recover when this obstruction is removed — an analogous state pertains in the kidney when obstruction of the urine by stone exists.

Associated with this condition there was always a diminution in the total bile when compared with the normal flow as given by Ranke, who considers one thousand and fifty grammes as normal for a man of seventy-five kilograms weight, while here the amount in the first instance was four hundred and fifty-one cubic centimeters average for the first two days, and four hundred and two cubic centimeters for the second day. Pfaff and Balch observed a daily flow of five hundred and fourteen and three-tenths cubic centimeters in a similar case of biliary fistula. Possibly this lessened secretion which, in the instances observed by me, was largely due to the diminution in water, may be due to dilated stomachs which, as is well known, cause such a diminution in the flow of urine. This condition is, not alone perhaps, but important in causing a concentration of all secretions, as evinced by the dry mouth and viscid saliva. Further, we may regard a delayed flow of bile through the finer biliary ducts in the liver as responsible, by which an opportunity for reabsorption of the water is permitted. One of the most remarkable changes, and one which pertains to both biles, is

the marked diminution of the cholalic acid. I could find no statistics in regard to estimations of this substance alone, so I was obliged, as stated above, to calculate it from the two cholates which are separately estimated in Hammarsten's article. This alone shows to what extent this acid was lacking and shows how it was possible for Hoppe-Seyler to find an entire absence of cholates in bladder bile taken at autopsy from sufferers from amyloid disease of the liver. As cholalic acid never appears in the urine and only to a slight degree in the feces, we must consider that it has an intermediate circulation like pepsin, though it has never been found in the blood, unless we accept Croftan's recently published work as final, an effort which must be repeated many times before we can consider this point established. Much to our surprise, we find no increase in the amount of cholesterin present. As the precipitation of cholesterin is so commonly a cause of gall stones, we must regard an abnormal increase of this substance, or diminution of its natural solvent, cholalic acid or the cholates, as a possible cause. Here we find the diminished cholalic acid as sufficient cause for the formation of these stones for which the operations were performed. Reabsorption of the cholalic acid, at least of the glycolate of sodium, according to Tappeiner, takes place in the jejunum; but whether such reabsorption might not take place in the biliary ducts of the liver, if the flow of bile through them was very much retarded, we do not know; it is a matter well worth further investigation. The solvent power of cholalic acid alone, apart from its association with sodium, is also an unknown quantity. Cholalic acid, as is well known, combines closely with albuminous material; and if in acid intoxication, a condition which in the obese is often associated with gall stones, the sodium is withdrawn, the combination of albumen and cholalic acid may fail to keep the cholesterin in solution. As certain alkaloids combine with cholalic acid and are thereby rendered temporarily innocuous, another possibility is presented that in serious disturbances of metabolism other abnormal products are formed which may unite with cholalic acid. Another unexpected result

was the marked increase in lecithin in the first case. I can find no record where this substance has ever been found in gall stones, but at least from its forming an important constituent of cells, particularly nerve cells, this increase means a severe disturbance of metabolism, which would take the formation of gall stones from the limited field of local inflammation of the biliary tract and make it more a general disturbance of nutrition. The calcium was not found increased in either case as compared with Jacobsen's analysis, whose relative amount of sodium to calcium was much less, viz.: thirty-five and four tenths to one. Hence it can be seen that there is no abnormality in the relation between calcium and sodium. The difficulties in quantitative determination of the taurin and glycocoll, as such, were so great that the results were of no value and are omitted. Hammarsten states that sulphur exists in other organic combination than that of taurocholate of sodium, so that the comparatively easy method of determining the sulphur and estimating the taurin from that is not available. The method of benzoyling the glycocoll and estimation of the hippuric acid formed may be made effectual, in the way that Spiro has used it, quantitatively, but in my hands it was futile, perhaps on account of the small quantities of glycocoll present. The essential features, then, of the results of this investigation are the moderate diminution in the solid residue found, the marked diminution of the cholalic acid, and the increase of lecithin found in the first instance. With the remainder of the bile which I have left, I am making some investigation in regard to the presence of ethereal sulphates and the bodies with which the sulphuric acid is paired, the results of which I expect to report in a later communication.

ACID-PROOF BACILLI IN FIVE CASES OF PULMONARY
GANGRENE.

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In the course of the last three years I had opportunity to observe five cases of pulmonary gangrene, the bacteriological and anatomical findings in which seem to be of some interest. The following is a short summary of these cases:

Case I.—Man thirty-seven years of age, with paresis and atrophy of right side of body due to a syphilitic tumor in right side of medulla oblongata. Suffers from cough with considerable sputum, night sweats, diarrhea for three and three-quarter months, during which time he reaches an extreme degree of emaciation. The post-mortem reveals — chronic putrid bronchitis, bronchiectases, chronic interstitial pneumonia with small abscesses at the end of dilated bronchi in right upper lobe, numerous more recent areas of chronic broncho-pneumonia with beginning carnification in both lower lobes. The central parts of the bronchopneumonic areas are gangrenous.

Bacteriological examination by cultures and suitably stained sections shows the presence in the diseased areas of staphylococcus pyogenes aureus, streptococci (none in cultures), and slender, often more or less curved, irregularly staining bacilli and short threads, with true branching. The organisms are frequently arranged in dense clusters and stain poorly in methylene blue, better in dilute carbolfuchsin, well with Gram's method. In coverslips and sections stained with Ziehl's carbol-fuchsin and then treated with one per cent hydrochloric acid solution in sixty per cent alcohol, they are decolorized; when the sections, however, are decolorized with sulphuric acid (twenty-five per cent) and mounted in glycerin in order to avoid the alcohol, they remain stained. The

staining is even better when weaker sulphuric acid solution (twelve and one-half per cent) is used. Unfortunately coverslips were not stained with the latter method. These bacteria did not grow on slanting glycerin-agar-agar under aërobic or anaërobic conditions.

The colonbacilli and yeast fungi which were also found in the cultures, but which could not be demonstrated in the sections, are with all probability to be regarded as contaminations.

Case II.—Old man, mill hand, seventy years of age, suffered from, according to his own statement, usually dry cough and some diarrhea for four months, lost in weight during that time. At the post-mortem we found a very marked anthracosis of the lungs and of the peribronchial lymphglands, with chronic inflammation of the latter. One of the anthracotic lymphglands on the right side had become necrotic and had perforated into a bronchial tube in the lower lobe, to which it adhered. The necrotic material had become gangrenous. The result was the formation at the hilus of the lung, in the diseased lymphgland and the adjoining pulmonary tissue, of a gangrenous cavity of the size of a cherry from which gangrenous material was discharged constantly into the bronchial tubes and gave rise to a chronic putrid bronchitis and tracheitis followed by chronic pneumonia of the right middle and lower lobes and chronic adhesive pleurisy on the right side with more recent catarrhal bronchopneumonia of the left lower lobe. In both apices there were old tubercular scars with old adhesions around them. There was also a chronic interstitial nephritis, with slight hypertrophy of the left ventricle, a chronic gastritis, and a beginning adenomatous hypertrophy of the prostate.

Bacteriological examination revealed in the gangrenous cavity and in the right lower lobe a mixture of streptococci and bacilli resembling very much those found in Case I. and in the left lower lobe (sections only) a few diplococci which resemble pneumococci.

The bacilli in the cavity grew on the glycerin-agar-agar in the form of minute moist grayish-white colonies for the first

generation, but no further growth could be obtained either under aërobic or anaërobic conditions.

Case III. — Laborer, fifty-two years old, suffered for five weeks from cough, hectic fever. Cough is accompanied with profuse fetid expectoration. Patient fails rapidly in strength and reaches an extreme degree of emaciation. The post-mortem revealed the existence of one large (size of fist) gangrenous cavity in the posterior part of the upper right lobe, several small ones in the anterior lower part of the same lobe, and also in the posterior part of the lower lobe. All cavities are surrounded by extensive areas of chronic catarrhal pneumonia with carnification. There was an old adhesive pleurisy on the right side, a few adhesions in the left pleura. The left lung was very emphysematous (compensatory emphysema). The left lower lobe showed a recent diffuse catarrhal pneumonia followed by acute pleurisy and acute pericarditis.

The bacteriological findings in cultures, coverslips, and sections were the following:

The contents of the large cavity consist almost entirely of pneumococci and bacilli very similar to those found in Cases I. and II. There are, however, some exceptionally large rods. On the wall of the cavity the organisms grow in the form of S-shaped bunches very much in the way of tubercle bacilli. The same mixture of bacteria was found in the bronchi and infundibula of the areas of chronic pneumonia surrounding the cavities, and in the recent catarrhal pneumonia in the left lobe.

The few colonies of colon bacilli which appeared in the cultures from the large cavity and the three colonies of staphylococci in cultures from the left lower lobe are very likely contaminations, since bacteria of this character could not be demonstrated in the sections.

Again, the bacteria showed some resistance against acid decolorizing agents in sections and did not grow on ordinary culture media.

Case IV. — Woman twenty-four years of age, suffering from epileptic attacks perhaps caused by the presence of cysticerci

in the motor areas of the brain, complained for over seven and one-half months of cough, night sweats, much loss in weight. The patient had already been at the hospital some years before the last admission on account of pulmonary trouble which then was diagnosticated as pneumonia and tuberculosis. Two and a half months before she died, a large abscess cavity in the left lung was opened, after resection of two ribs. The patient died in an extremely emaciated condition. At autopsy the left lung was found honeycombed with cavities full of gangrenous contents, the largest one of which was the size of an English walnut. The pulmonary tissue between the cavities was in a state of subacute and chronic catarrhal pneumonia with carnification. The left pleural cavity was obliterated by strong adhesions. The right lung in the lower lobe showed a compensatory emphysema and broncho-pneumonic patches with central necroses.

Bacteriological examination of cultures, coverslips, and sections reveals the presence in the diseased areas of pneumococci and acid-proof bacteria very much like those found in the other cases, but which did not grow on artificial culture media.

Case V. Acute gangrene.—Man of fifty-two years, in good health; in a railway accident he received a severe fracture of the skull, which was treated surgically. Patient remained unconscious and died not quite five days after receiving the injury. He had fever which on the day before his death reached 105° F.

At the autopsy the brain showed a traumatic hemorrhagic softening of both temporal lobes. There was a recent thrombosis of the left sigmoid sinus, an embolus in one of the large branches of the pulmonary artery, a recent pneumonic consolidation in the area of pulmonary tissue to which the obstructed branch of the pulmonary artery led, with central diffuse gangrene; gangrenous pleurisy on the right side, acute broncho-pneumonia in the left lower lobe.

The diseased areas contain pneumococci and bacilli very much like those in the four cases of chronic gangrene. The colon bacilli which were found in the cultures from the gangre-

nous area, the broncho-pneumonic patches, the spleen, and the liver could not be demonstrated in sections. It is very probable that they were accidental contaminations.

We have then five cases of pulmonary gangrene, four chronic and one acute one, in the lesions of which bacilli were found, which show the following characteristics:

They are long, slender, more or less curved rods or threads with irregularly staining protoplasm, they frequently occur in clusters; sometimes these bunches are more or less S-shaped. In all cases it was possible to find individuals which showed true branching. These bacteria stain badly with methylene blue, better with dilute carbol-fuchsin, very well with Gram's method, which brings out their irregular staining particularly clearly. After having been stained with Ziehl's carbol-fuchsin the bacteria decolorize in acid alcohol, but not in aqueous solution of sulphuric acid (twelve and one-half per cent). Except in Case II. attempts at cultivation failed, and even in this case a very scanty growth was obtained in the first generation only and there was no further development.

In the absence of pure cultures it is impossible to decide whether the bacteria in all five cases belong to the same species. The presence of certain variations in form (some particularly large individuals in Case III., many S-shaped individuals in Case II.) might induce one to be doubtful in this regard. However, it is well known that bacteria of this kind show a marked tendency to variation. The bacteria certainly are very closely related to one another, and quite apparently belong to the acid-proof bacilli such as occur on timothy grass, in butter, etc., although they deviate from them in certain particulars, not growing well on artificial culture media, being perhaps less acid proof, and so forth.

It seems that Fränkel¹ was the first to call attention to the occurrence of acid-proof bacilli in pulmonary gangrene. The same observation was made apparently independently in 1898 by Pappenheim,² who believed the bacteria which he found to be smegma-bacilli. In 1884 Zahn³ recorded the occurrence of acid-proof bacilli in the sputum of pa-

tients not suffering from tuberculosis. These earlier reports were confirmed by Rabinowitsch.⁵ Rabinowitsch, in 1900, found acid-proof bacteria in one case of pulmonary gangrene in the sputum and also in the tissues at the post-mortem. She obtained pure cultures in which the bacteria grew in the form of an orange-colored wrinkled membrane. They were pathogenic for mice in a way similar to the acid-proof bacilli found in butter. She believes, however, that the bacteria found in gangrene and those commonly occurring in butter are, although closely related, yet different species, largely on account of their somewhat different growth on artificial culture media. In the same year Benvenuti⁶ found in the sputum of a case of pulmonary gangrene acid-proof bacteria which during the lifetime of the patient were mistaken for tubercle bacilli. The post-mortem, however, showed a complete absence of tubercular lesions, and animals inoculated with material obtained at the autopsy did not become tuberculous.

Even more interesting are some observations made by Mayer,⁷ a German military surgeon, in 1901, while he was stationed in China. Among his patients he found an unusual number of cases of pulmonary gangrene, and among fifty-eight cases in which the sputum was examined for tubercle bacilli he found in ten acid-proof rods which were not tubercle bacilli and which he classifies as streptothrices. In one of these cases he was able to make a post-mortem, found a circumscribed gangrene of the right upper lobe, and demonstrated the bacteria in the tissues. He also obtained pure cultures from the sputum in several cases in which the organisms grew in the form of a white — later colored — wrinkled membrane. They grew well on artificial culture media, even at room temperature. Animal experiments were negative. Similar bacteria were encountered by him in a pelvic abscess in a Chinese. He refers briefly to similar findings by Aoyama and Miyamoto, two Japanese authors, of which I have not been able to find any record in the literature which was accessible to me. Mayer concludes his letter with the following significant remark: "Es ist wohl nicht aus-zuschliessen

dass hier eventuell das Bild einer 'primaeren Lungengangraen' durch obige säurefeste Streptothrix vorliegt."

Rabinowitsch, Benvenuti, and Mayer succeeded in cultivating the organisms which they found, whereas my attempts at cultivation have been unsuccessful. I believe, however, that my failure may be ascribed in part to the limited time which I could devote to these cultivation experiments. If I had been able to choose my media carefully and use other precautions which I had to neglect, I might have been able to overcome the difficulties which we usually encounter in obtaining the first growths of organism of this kind on artificial culture media.

I believe one more, rather early, observation belongs in the same category of cases. I mean one recorded in a paper on the "etiology of acute pulmonary destruction" by Buchholtz⁸ which seems to have escaped the attention of other authors on account of its somewhat misleading title. The case described in the paper is one of pulmonary gangrene of both lower lobes with chronic pneumonia. Buchholtz found streptococci and a "streptothrix," which, however, does not seem to have been very carefully investigated in regard to the effects of acid decolorizing fluids on its staining. Cultures were not obtained. Buchholtz believes that the streptothrix was the cause of the disease and the streptococcus infection was secondary. I am also reasonably certain that Babes,²⁰ who has made very careful bacteriological investigations on a comparatively large material, has seen similar bacteria. In ten out of twenty-four cases examined he found bacteria which he describes as "dipteridés," i.e., similar to diphtheria-bacilli, which proves that they belong at least to the same genus.

All these observations tend to show that in pulmonary gangrene one frequently finds more or less acid-proof bacilli of the class of streptothrices or perhaps more correctly actinomyces, and it is certainly a very suggestive fact that in all five cases of pulmonary gangrene which I had the opportunity to examine more carefully such bacteria could be easily demonstrated.

Of other parasitic organisms found in this disease we have a number of early reports from the time when the occurrence of living parasitic organisms in pathological lesions first attracted the attention of medical observers. Virchow⁹ and Cohnstein¹⁰ describe the occurrence of sarcinæ and mould-fungi, Rosentein¹¹ encountered *Oidium albicans*, Leyden and Jaffé¹² leptothrix and spirilla; the latter also claim to have seen peculiar infusoria, in which observation they were confirmed by Kannenberg¹³ and Streng.¹⁴ More recently Bonomé¹⁵ examined eight cases of pulmonary gangrene, of which six were of an embolic nature bacteriologically, and found in all cases either *staphylococcus pyogenes aureus* or *albus*. He believes that the infection with pyogenic cocci is the cause of the gangrenous process. He even claims to have produced gangrenous areas in the lungs of rabbits by direct traumatism of the lungs with instruments which he had soiled with cultures of staphylococci. Cultures from the gangrenous areas always contained the pyogenic cocci used in the experiment. Bonomé does not state, however, whether they were present in pure culture. I believe that in his paper Bonomé does not distinguish sharply enough between necrosis and gangrene. Nobody will doubt that staphylococci can cause necrosis and in that way prepare the tissues for gangrene, but I do not think that many will concede that pyogenic cocci unaided can cause gangrene. Hirschler and Terray¹⁶ isolated a new variety of staphylococci from the sputum and the gangrenous tissues of three cases of pulmonary gangrene. Cultures of this coccus have the characteristic odor of gangrenous sputum, and the authors even claim to have reproduced typical pulmonary gangrene by direct inoculation of pure cultures into the pulmonary tissue of animals. The records of their animal experiments, however, are not entirely convincing. Lumniczer¹⁷ described a case in which he found leptothrix, spirilla, pyogenic cocci, and a curved motile spore-forming bacillus. Cultures of the latter had the characteristic odor of the sputum of the patient. When, on improvement of the latter's condition, the bad odor disappeared from his sputum, the bacillus also disappeared, to

reappear when later the odor returned to the sputum. According to Lumniczer these bacilli were pathogenic for animals and produce (in conjunction with pyogenic cocci) inflammation and necrosis, but, as far as one can recognize from the records of the experiments, no gangrene. Barnabei and Alfieri¹⁸ found bacilli of the colon-group in cases of pulmonary gangrene. Reinbach¹⁹ from Ziegler's laboratory publishes a case of gangrene of both lower lobes in a child in which bacilli were present which closely resembled anthrax-bacilli. Babes²⁰ reports several cases in which he found bacilli resembling the bacillus of malignant edema.

In the *Centralblatt für Bacteriologie und Parasitenkunde* (xxx. s. 281, 1901) Rist gives a synopsis of some recent French investigations into the etiology of pulmonary gangrene, which, he claims, demonstrate that this disease is always due to an infection of the pulmonary tissue with anaërobic bacteria. He acknowledges that one usually finds some aërobic bacteria such as certain "non-pathogenic" streptococci, more rarely staphylococci, *B. proteus*, *B. coli communis*, but asserts that their number is very small compared to that of the anaërobs present, among which a *Bacillus ramosus* first described by Veillon and Zuber seems to play the most important rôle. This bacillus is a slender rod which, when found in pus, is short, on artificial media long, thread-like; it is non-motile, strictly anaërobic, remains stained when treated according to Gram's method, grows at 37° C. only, the growth resembling that of streptococci. In the cultures it produces little gas, which, however, has an extremely offensive odor. From its name one would suppose that the bacterium showed branching. Rist does not state this, however. Guillemot, whose researches Rist reviews, succeeded in producing embolic pulmonary gangrene in rabbits and guinea-pigs by intravenous injection of cultures of *Bacillus ramosus* together with other anaërobic bacteria. As I have stated before, I did not succeed in obtaining cultures of the bacteria found in my cases under anaërobic conditions; otherwise the bacteria present in my cases might have some similarity to the *bacillus ramosus*, if the latter really should show

branching as the name implies. Other bacteria apart from the pyogenic cocci were certainly not present in any of my cases except for occasional contaminations.

When we come to ask ourselves what is the significance of the acid-proof bacilli in those cases of pulmonary gangrene in which they are present, we might feel inclined to regard them either as entirely saprophytic organisms which find a suitable culture medium in the gangrenous tissues, or to attribute at least some etiological importance to them. I believe that Buchholtz⁸ goes too far when he assumes that the streptothrix which he found was in his case the cause of the pulmonary gangrene, and that the streptococci which were present also only played the part of secondary invaders. We know that streptococci are pathogenic and, therefore, as long as there is any doubt it is safest to attribute as much of the pathological process as we possibly can to their pathogenic activity. The mere fact that these acid-proof bacilli have always been found in association with other undoubtedly pathogenic bacteria (in my cases, for instance, with pneumococci, streptococci, staphylococci), a fact which is particularly emphasized by Babès, to my mind also suggests strongly that we should not ascribe to them pathogenic power in the ordinary sense of the word. If they had the power to develop in healthy tissue and cause disease, it would be remarkable that they have not once been found alone in the lesions. One might object that our experience in pulmonary phthisis demonstrates the frequency if not constancy of secondary and mixed infections with pyogenic cocci in the lungs, but my investigations have convinced me that simple infections of the lungs with tubercle bacilli certainly do occur, and not so rarely as one might think. I have also found bacteria very much like those present in these cases of pulmonary gangrene in other abscesses with stinking pus, but in all cases they were associated with pyogenic cocci. On the other hand, pyogenic cocci are not known by themselves to produce gangrene. It is true that such has been asserted by Bonomé in a more general way,

and by Hirschler and Terray for a special form of staphylococci which they cultivated from gangrenous tissues, but from all we know about the pathogenic action of pyogenic cocci it would take stronger evidence than is presented in these papers to carry conviction with it to my mind. Pyogenic cocci certainly can produce necrosis, but hardly gangrene. It seems to me most probable, therefore, that the pathological alterations which we observe in these cases of pulmonary gangrene are brought about by the pyogenic cocci and the acid-proof bacilli conjointly in such a way that the pyogenic cocci injure the tissues to such an extent that the acid-proof bacilli can develop in them, which then in their turn assist them in producing the necessary necrosis and then produce the liquefaction of the tissues into a discolored more or less fluid stinking material: the gangrene. I am all the more inclined to believe that such is the case because in a case of pulmonary tuberculosis complicated by gangrene, in another case of nearly healed tuberculosis with chronic fetid bronchitis, and in a case with a foreign body in one of the main bronchi on the right side and with secondary tuberculosis and gangrene of the right lung, I have been able to find similar bacteria in the gangrenous material. It is well to emphasize, however, that there is a possibility that the bacteria are chance saprophytic inhabitants of the gangrenous material, and that the gangrene is produced by organisms which we cannot demonstrate with our present methods.

Experiments with pure cultures alone could decide this question definitely, but my failure to obtain cultures of the acid-proof bacilli in my cases made such experiments impossible for me.

I strongly suspect that the acid-proof bacteria come from the mouth, because in several cases of suppuration following disease of the teeth and gums in which the pus had an extremely unpleasant odor similar organisms were seen in the coverslips from the pus. As a confirmation of this opinion the findings of Laabs⁴ may be mentioned, who discovered acid-proof bacilli in the saliva, coating of the tongue and teeth of individuals apparently not suffering from tuberculosis.

In most text-books it is expressly stated that pulmonary gangrene is a rare disease. Here in San Francisco I counted, among approximately five hundred autopsies, ten cases with a gangrenous condition of the lungs, which would seem to indicate that the disease is more frequently found here than elsewhere. It was interesting for me to read that Mayer reports the same from the other side of the Pacific Ocean.

In three of our cases a definite predisposition for the gangrenous process was given (in Case I. interference with respiration by paralysis of the right side of the body, chronic putrid bronchitis; in Case II. perforation of a necrotic anthracotic peribronchial lymphgland; and in Case V. circulatory disturbances due to embolism in a branch of the pulmonary artery). In the two other cases such predisposing moments are not evident. Both present the picture of a chronic catarrhal pneumonia with partial organization of the exudate and gangrenous cavities in the middle of the affected areas. I believe it would be well to separate such cases from the more common ones of secondary gangrene and call them, with Mayer, cases of "primary pulmonary gangrene," although etiologically and histologically, as one can see from the records, they do not differ from one another.

Conclusions.

1. Pulmonary gangrene is very probably always due to mixed infection with pyogenic cocci and other bacteria of a more saprophytic nature.
2. Quite frequently the latter belong to the class of actinomyces (including in this class all branching bacteria like tubercle bacilli, diphtheria bacilli, etc.), and they may be more or less acid-proof.
3. The gangrenous process is always accompanied by pneumonic processes which in the more chronic cases usually appear in the form of a chronic catarrhal pneumonia with carnification.

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TRICHINOSIS.

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During the past few months I have had an opportunity of studying four cases of trichinosis among patients and an opportunity of studying trichinosis in a herd of swine of two hundred in which trichinosis was quite prevalent. An opportunity was given me of making numerous blood examinations in trichinous swine, of making autopsies in a number of the same; of beginning a series of experiments, feeding animals on cooked trichinous pork; of attempting to solve the question of a practical method of separating the trichinous swine from the living non-diseased, which would appear at first difficult, as outwardly the diseased swine show no evidence of trichinosis; and we have often found the largest and best developed riddled with trichinæ throughout its muscles.

I shall also report repeated blood examinations showing no eosinophilia in four cases of trichinosis in human beings.

The literature of trichinosis is abundant and covers a period of about seventy-five years. It was an American who first discovered what he described as "specks" in a ham sandwich he was eating. Germany has apparently suffered the most from trichinosis, where serious epidemics of the disease can be quoted, namely: a village of two thousand people had three hundred and thirty-seven cases of trichinosis within a short period of time, with one hundred and one deaths resulting; another town of one thousand six hundred inhabitants — the flesh from one hog is said to have given rise to two hundred and fifty-six cases, with fifty-six deaths resulting.

Such figures indicate that the pork must have been eaten nearly raw or at least imperfectly cooked. There are no

records of such epidemics in this country, where we cook our food more thoroughly.

To quote from the Department of Agriculture Records: "The consumer can protect himself absolutely from this disease by requiring all pork to be thoroughly cooked. Cases of trichinosis in this country occur from eating fresh pork or insufficiently cooked, and sometimes not cooked at all. Were it not for our German population trichinosis would hardly be worthy of consideration as a sanitary question. However, the elaborate and expensive system of inspection adopted by Germany has not fulfilled expectations, as the number of cases of trichinosis from inspected pork is so large that it rather indicates the unreliability of the method than inspires confidence as a prophylactic measure."

This brings us to the subject of government microscopic meat inspection which was given up by our government on Jan. 1, 1902.

In 1881 Germany and France, who inspect their own meat, prohibited the entrance of American meat to their markets, and thus a large American export trade was shut out for ten years, or until 1891, when our government began a systematic microscopic examination of pork destined to the prohibiting countries, and the export trade was resumed. In the first year (1892) one and one-fourth million hogs were inspected and twenty-five thousand, or two per cent, were found trichinous; which means that the non-trichinous hogs were exported and the remainder saved for our own markets, taking the precaution of thoroughly cooking the latter.

During the past ten years one to two million animals have been exported annually and from two to three per cent found trichinous. The average cost of inspection is six cents a head. Our export trade is again prohibited by Germany, as we no longer inspect our pork.

In 1900 pork was inspected in forty-five localities, but there was a lessened exportation of American pork, due doubtless to increased price in our home market.

Trichinosis has been supposed to be a rather rare disease in America, whereas in Germany, from 1883 to 1891, there

was an annual average of four hundred and fifty-four cases. According to Williams, up to 1898 only nine hundred cases in man are reported as occurring in the United States. Williams also reports his findings in five hundred and five autopsies as five and three-tenths per cent trichinous.¹

Of two hundred and seventy-four cases of trichinosis in this country, seventy-six per cent were of German birth.

Trichinæ are also found in cats, rats, and dogs, and other flesh-eating animals.

If trichinous pork is taken into the stomach, the capsules are dissolved, freeing the trichinæ, a single embryo giving rise to one thousand five hundred to two thousand, producing gastroenteritis a few days after the ingestion of the pork. The young trichinæ find their way presumably by the lymph stream to the muscles, where they are encapsulated shortly. A myositis is produced with the well-marked symptoms of pain, swelling, œdema, and partial paralysis; the muscles of larynx and mastication may be involved to an extreme degree, causing difficulty in speaking and swallowing. For some unknown reason the heart muscle alone escapes.

Two or more trichinæ may be surrounded by one capsule. A great many may be discharged by the intestines, and a practical method of diagnosis is to examine the discharges for trichinæ, which appear as fine white thread-like worms just visible to the naked eye.

Since blood examinations have taken so prominent a place in the diagnosis of many diseases, eosinophilia has been regarded as a diagnostic sign of this disease.

In the "Journal of Experimental Medicine," 1898, Brown arrives at the following conclusions from three cases of trichinosis studied in Osler's clinic:

I. "That there is a marked increase in trichinosis in the per cent of eosinophilic cells in the blood."

II. "That this increase may be used as a diagnostic sign in this disease."

III. "That this disease in its sporadic form is more common than has been hitherto supposed, as shown by the dis-

¹ July, 1901, The Journal of Medical Research, Vol. vi, No. 1.

covery of the three cases within a comparatively short period of time at the Johns Hopkins Hospital."

IV. "That a systematic examination of the blood should be undertaken in cases with indefinite intestinal symptoms, muscular or articular symptoms, in the hope that in some, at least, of the hitherto doubtful cases, a diagnosis may be reached."

Since the publication of this paper, about twenty-five cases have been reported with full blood examinations, and all but one (a case reported recently in the "American Journal of Medical Sciences," by Da Costa) showed a well-marked eosinophilia. Later in this paper I shall present four cases with trichinæ present in muscles and having marked muscular tenderness, edema, and enteric disturbances, with a normal proportion of eosinophiles present in the blood—which leads us to conclude that there is no one diagnostic sign of trichinosis. Eosinophilia occurs also chiefly in scarlet fever, in other animal parasitic affections beside trichinosis, in severe anemias and blood diseases, bronchial asthma, malignant disease, and in eczema, dermatitis, and allied skin diseases.

Coplin in his discussion of Da Costa's case with no eosinophilia suggests that it may be practically always present in the initial outbreak of the disease, but not present in its recrudescences.

Trichinosis among the swine at the Northampton Insane Hospital.

"Prof. E. L. Mark, of Harvard University, in the Report of the Massachusetts State Board of Health for 1894, gives his results of examinations for trichinæ in pork at the above institution for the years 1884 to 1894 inclusive." A total of four hundred and forty-nine slaughtered hogs were examined in ten years and fifty-four were found trichinous. In 1887 a change in diet was made, cooked kitchen offal was substituted for uncooked, and the slaughter house refuse excluded; there resulted an almost yearly diminution in per cent of trichinous hogs. The following percentages: in 1887,

nineteen and three-tenths per cent; 1888, fourteen per cent; 1889, seventeen and one-tenth per cent; 1890, ten and two-tenths per cent; 1891, three and nine-tenths per cent; 1892, three and seven-tenths per cent; 1893, two and seven-tenths per cent; 1894, none; 1895, none.

No cases were reported among the patients.

For the past ten years a systematic examination of pork raised at the State Hospital has been made. About ten years ago a few cases of swine trichinosis were found in specimens examined by Professor Mark, to whom specimens of all slaughtered swine were sent.

For the past four years this examination has been done by the writer, and previous to a year ago no trichinous swine were found. The technique is very simple; a piece of fresh muscle smaller than a split pea is squeezed between two glass slides and immediately examined with a low-power microscope, as only the calcified or partially calcified forms are visible to the naked eye. In none of the pork specimens seen by the writer were they visible without microscopic examination. Specimens are taken from the diaphragm and pillars of the latter and intercostal muscles. Last fall an occasional positive result was obtained, and during the winter were found others with increasing frequency. This, with the discovery of a patient in the asylum wards afflicted with trichinosis, led to the following investigations.

When considering the means of eliminating trichinosis from our herd of swine, the suggestion was made that a blood examination might show an eosinophilia in the afflicted swine as in patients. Cover-glass preparations were made from one hundred individual hogs with negative results excepting marked leucocytosis in two cases, autopsies of which showed lung tuberculosis. There was left apparently one method of procedure, viz., excision of muscle of live swine. By experimenting on hams the location was determined where the muscle was directly beneath the skin, so that only a three-fourth-inch incision would show the desired muscle and a piece the size of a pea could easily be excised. All the incisions healed quickly with no ill results. During the

operation the animal was placed on its back, each assistant holding an extremity. In five hours' actual work with one microscope, ninety were examined. In this way one hundred and seventy-eight were examined and nineteen found trichinous, or ten and seven-tenths per cent of the entire herd of swine. The condemned swine were later autopsied and the muscle previously examined was, as a rule, found as thickly sown with the trichinæ as any other portion of the carcass excepting the diaphragm. The tongue was almost invariably infected. None were found in the heart muscle. The fat only of the above nineteen was made use of. This apparent waste would seem justifiable, as I shall quote cases apparently caused by supposedly thoroughly cooked pork.

There were several pens of sixteen to eighteen swine in which no trichinæ were found; on the other hand, as a rule, three to four cases were frequently found herded together in a pen. The crowding together of large numbers would seem to favor the spread of trichinosis.

During the winter months the swine are kept in pens numbering fifteen to twenty in each, but during the summer months are allowed more than an acre of pasturage.

We do not expect all the mild cases among the swine were found and condemned on this first examination, as in certain instances repeated examinations of many muscles of a carcass are necessary before a single trichina is found. By thoroughly boiling all articles of food fed the swine, the exclusion of slaughter-house refuse, by cleansing the pens, and by the above process of elimination at intervals of as many diseased swine as possible, a decrease in trichinosis among our swine in the near future is anticipated.

The following blood examinations were made at the time of slaughter from fifteen normal and fifteen trichinous swine, showing a varying per cent of eosinophiles which on the average in these cases was found one per cent lower in trichinous than in non-trichinous swine:

Positive.

No. Specimen.		Per cent Lymphocytes.	Per cent Polynuclear.	Per cent Eosinophiles.
2	Positive.	62.	37.5	0.5
8	"	60.	35.	5.
11	"	68.	29.	3.
13	"	65.	32.	3.
16	"	72.	26.	2.
18	"	67.	32.	1.
22	"	53.	42.	5.
26	"	62.	32.	6.
29	"	60.	35.	5.
23a	"	66.	33.	1.
24a	"	71.	25.	4.
25a	"	61.	29.	10.
27a	"	66.	31.	3.
28a	"	56.	38.	6.
30a	"	60.	34.	6.
15 examined. Average		63.26+	32.7	4.03

Negative.

No. Specimen.		Per cent Lymphocytes.	Per cent Polynuclear.	Per cent Eosinophiles.
1	Negative.	60.	38.	2.
3	"	67.	29.	4.
4	"	60.	35.	5.
5	"	51.	37.	12.
6	"	52.	44.	4.
7	"	66.	33.	1.
9	"	57.	39.	4.
10	"	70.	24.	6.
12	"	46.	44.	10.
14	"	65.	30.	5.
15	"	33.	66.	1.
17	"	55.	39.	6.
19	"	77.	18.	5.
20	"	51.	43.	6.
21	"	36.	58.	6.
15 examined. Average		56.40	38.46+	5.13+

From these examinations we conclude that there is in swine trichinosis no increase in the per cent of eosinophiles. I wish to now briefly call your attention to five clinical cases of trichinosis. In four cases I have made complete examinations also showing no eosinophilia.

John H., born in Massachusetts, fifty-one years of age, a farm laborer, was admitted to the State Hospital from Lawrence, November 26, 1900. He was somewhat demented, but stated that he had wandered about for one month, unable to work, and the past week had been in the Lawrence Hospital, his chief complaint being weakness. He was unable to walk, his appetite was poor, slept poorly.

December 9. Temperature rose from normal to one hundred and two and five-tenths. Pulse one hundred and five. Respiration forty. Has had diarrhoea during past twenty-four hours and died at 8.35 P.M.

A few days before death the diagnosis of trichinosis was considered and with this in view a blood examination for eosinophilia was requested, which unfortunately was not made. An autopsy was obtained at which time post-mortem changes made a blood examination impossible.

Large numbers of trichinæ were found in every specimen of muscle taken from diaphragm, biceps, pectoral, jaw-muscles, and tongue. None were found in the heart muscle.

James M., born in Ireland, age thirty-one years. A case of dementia. For the past ten years a constant inmate of the asylum wards of the State Hospital and in good physical condition up to December 30, 1901, when he was taken with a chill. Temperature, 104° F. Pulse, one hundred and twenty-five. Respiration, 28. With an attack of vomiting.

February 12. Blood examination showed Widal negative. Red count three million seven hundred and four thousand. White, six thousand six hundred. Polymorphonuclear neutrophiles, sixty-five per cent. Large and small lymphocytes thirty-four and two tenths per cent. Eosinophiles eight-tenths.

A piece of muscle was removed from the calf of leg and numerous encysted trichinæ were found, eight or ten being

found in a microscopic field with low power, or AA objective and No. 2 eyepiece.

The patient developed progressive weakness with rising pulse and respirations, with at times slight hacking cough. No expectoration. Extreme soreness of muscles, and died March 6, 1902.

Three additional differential white blood counts are recorded, made during the last month of his sickness, but at no time was more than one per cent of eosinophiles noted.

At the autopsy all the muscles appeared as thickly and diffusely sown with grayish bodies resembling particles of sand plainly visible to the naked eye. Microscopic examination showed the above to be encapsulated trichinæ. Trichinæ were found in great numbers in the tongue and larynx. None were found in the heart muscle, liver, spleen, kidneys, muscles of intestines, or lymphatic glands or lungs. No trichinæ were found in intestinal contents. The lungs were voluminous and sown throughout with discrete miliary tubercles. Microscopic examination of scrapings from the surface showed numerous tubercle bacilli.

Additional Blood Counts.

	Sm. Lymph.	Large Lymph.	Transit.	Poly-morpho.	Eosino- philes.
2-11-02	14.	2.	0.0	83.5	0.5
2-24-02	20.	4.	1.0	75.	0.0
2-26-02	16.	2.	0.0	82.	0.0
3- 2-02	12.	2.5	0.5	84.5	0.5
3- 6-02	14.	5.	0.0	81.	0.0

Iodophilia, positive reaction.

Abbie S. Age, thirty-five years. Born in Massachusetts. An inmate of the institution for three years, was admitted to the hospital wards on January 22, 1902, with the history that for the past two and one-half months she had at times refused to walk till at present she gets about on her hands and knees, saying that she "is sick and cannot walk."

A diarrhea became more marked later, and the muscular tenderness with edema and absent reflexes persisted so that

on February eighteenth a small bit of muscle from the calf of the leg was taken out under cocaine, and this showed encapsulated trichinæ present in considerable numbers, *i.e.*, approximately one in every two to three fields with the lower power (AA obj. and No. 1 eyepiece).

Her symptoms have remained unchanged except loss of flesh, which has been very great, having lost about one hundred and twenty pounds since entrance, January 22. Former weight two hundred plus.

She frequently cries out with pain in her extremities and the tenderness remains about the same, while she still has attacks of diarrhoea which last for a day or two at a time.

Her temperature on the day after admission was 102°, since which time it has been slightly above normal (99°), but only twice, however, reaching 100°. Urine negative. No Diazo.

Blood Examinations and Counts.

	Sm. Lymph.	Large Lymph.	Transit.	Poly-morpho.	Eosino- philes.
2-24-02	17.5	6.5	1.5	73.	1.5
3- 4-02	26.	2.	.5	70.	1.5
3-12-02.	30.	5.	2.	62.	1.
3-24-02.	17.5	4.5	1.	75.	2.
4-21-02.	27.5	2.	1.5	67.	2.
4-27-02.	21.	2.5	0.	76.	.5

White count, eight thousand eight hundred.

John P. Age fifty-four years. Born in Ireland. A case of dementia. Was admitted to the State Hospital November 21, 1898. Nothing unusual was noticed about this patient until December 25, 1901, at which time he was removed to the hospital wards for so-called general weakness. In February slight tenderness was noted. The blood examination was reported as being normal. A piece of muscle was excised from the gastrocnemius and no trichinæ found. The patient improved sufficiently to return to asylum wards March 3, 1902. March 27, 1902, was returned to hospital with diarrhea, temperature 101° to 102° F., and weakness of arms and legs.

April 16, 1902, this condition being unexplainable, a second piece of muscle was excised and this time a few trichinæ were found present.

The second incision was made in the same muscle as the first.

The urine showed no Diazo.

A trace of albumen.

With few leucocytes. No casts.

The following blood examinations made:

White count seven thousand.

	Sm. Lymph.	Large Lymph.	Transit.	Polymorpho.	Eosinophiles.
4-16-02.	24.	3.	0.	72.	1.
4-22-02.	22.	5.5	0.	72.	.5
4-27-02.	38.	3.	0.	58.	1.

Walter M. Age sixty-four. For past ten years a resident of Massachusetts. Was admitted to the State Hospital, February 18, 1902. Patient is an advanced case of phthisis.

Aside from his history of phthisis he also stated that for the past five or six months he noticed that his muscles were getting tender, and he had pains in the same and was unaccountably weak. Feet had not been swollen until the past month, and on the first of April the edema of lower extremities and the right arm was very marked, as was also local tenderness on pressure.

Under cocaine a piece of muscle was removed from calf of left leg and a partially calcified trichina found. This patient stated that he is fond of pork, but has eaten none during the past six months. Has had occasional attacks of diarrhea during the past six months, but is habitually constipated. The following blood examinations have been made:

White count nine thousand.

	Sm. Lymph.	Large Lymph.	Transit.	Polymorpho.	Eosinophiles.
4-17-02.	14.	5.5	.5	80.	0.
4-22-02.	9.	2.	1.	88.	0.

Animal Experimentation. — Preliminary Report on Animal Feeding.

Three cats and a like number of rats for six weeks were fed a considerable quantity of ham (which was previously pickled three weeks, smoked two days, then boiled constantly for five hours).

One of the animals, a cat bred in this Institution, showed at the autopsy an enormous number of recently encapsulated trichinæ throughout all its muscles. As the cat may have acquired trichinosis previously, no conclusions are drawn from the above experiment, but further experimentation with cooked trichinous pork will be reported at a later date.

Conclusions as Regards the Value of Eosinophilia in Trichinosis.

That it may not be present during the course of trichinosis, as shown by these cases and the case reported by Da Costa in "American Journal of Medical Sciences," 1901.

That although present in about twenty-five cases reported in this country at the initial outbreak of this disease, it may not be present during the course and recrudescences of the disease, even if the infection be a severe one.

That eosinophilia with certain other signs points to the diagnosis of trichinosis. That the absence of eosinophilia should by no means eliminate the possibility of trichinosis being present, and recalls the fact that no one sign is pathognomonic of a given disease. That it is practical in a great measure to separate trichinous swine from the non-diseased without losing the entire herd. That further animal experimentation is desirable to determine definitely when meat is sufficiently cooked for safe use. That trichinosis, complicated or not, may in mild cases be more common than is generally considered, and that Government inspection of pork would be a desirable procedure to continue for the general safety and public good, as shown by cases occurring in this Institution where well-cooked pork only is served. That trichinosis may be readily diagnosticated, or it may be a very obscure

disease, -especially if accompanied by phthisis, or disease in which enteritis or swelling of extremities may occur. That it may simulate typhoid fever, or muscular rheumatism, or peripheral neuritis. That there may be periodic attacks or recurrences resembling the relapses of typhoid fever, explained only by the theory that new crops are liberated either by a long hidden embryo in the intestine or encapsulated forms freed and in some way returned to the intestine.

A systematic examination of the patients in our asylum wards failed to show further cases of trichinosis. Particular attention was paid to patients with edema, muscular tenderness, or enteritis, with weakness, or paralysis. The blood of one hundred such cases showed no eosinophilia. In rare instances muscle was excised with negative results.

Three of the cases reported in this paper appear to have contracted trichinosis while in this Institution, notwithstanding our systematic examination of pork and what was supposed to be thorough cooking of the same. Government inspection we know, however, to not be infallible, and it would thus also appear from the evidence of the above cases that well-cooked pork may not be sufficiently cooked, as these patients have been under constant supervision and could by no possibility have access to raw pork.

In concluding I would advocate the more careful inspection of pork in our State Institutions, for trichinosis, for among the insane patients trichinosis has long been known to be unusually prevalent; would recommend our method of eliminating trichinous swine from others, and would advocate further Government inspection of pork, provided the infected meat was destroyed or rendered harmless.

DESCRIPTION OF PLATE XVII.

- FIG. 1. *Trichina* removed from capsule. Teased preparation.
FIG. 2. *Trichinæ* covered with granulations.
FIG. 3. *Trichinæ* in human muscle cleared with HCl. Fatal case.
(Photographs by Wright and Brown.)

THE MODE OF ACTION OF VARIOUS LAKING AGENTS ON
THE BLOOD CORPUSCLES.¹

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In a previous paper² I showed that the peculiarities in the behavior of dog's blood to NH_4Cl , NaCl , saponin and water, revealed by measurements of the electrical conductivity of mixtures of blood with these substances in definite proportions, are the same whether the blood be fresh or stale, provided that the corpuscles are intact. Even prolonged treatment with formaldehyde does not fundamentally alter the behavior of the blood in this regard. From these facts I deduced, among other consequences, the conclusion that the peculiarities in question, and among them the selective absorption of NH_4Cl by the corpuscles, are dependent not on their life but on their structure or chemical composition. Manca³ showed some time ago that the behavior of the corpuscles to NaCl and KCl , to both of which substances they are practically impermeable, and other properties, *e.g.*, their "resistance" to hypotonic solutions of NaCl , are not altered when they are kept for a considerable time, when they are acted on by chloroform, etc.

In pursuing the subject I have been led to compare the action of a number of hemolytic agents on fresh and hardened blood, and have arrived at certain results as to the *modus operandi* of these agents, which, although still incom-

¹ Many of the results here recorded were communicated to the American Physiological Society at the Chicago meeting in December, 1901, and the rest to the American Association of Pathologists and Bacteriologists at the Cleveland meeting in March, 1902. Some of the experiments (including those on ether-extracted formaldehyde corpuscles, on the laking of formaldehyde corpuscles, and on sublimate-fixed corpuscles) were made in conjunction with Dr. S. Peskind, H. M. Hanna Research Fellow in Western Reserve University.

² Jour. of Physiol., xxvi, p. 470.

³ Arch. ital. de Biol., t. xxiii, 1895, p. 317, 391; Ibid. t. xxix, 1898, p. 342; Ibid. t. xxx, 1898, p. 78. Archivio per le Science Med., t. xx, 1896.

plete, it seems desirable to publish now. In addition to saponin (including sapotoxin) and water I have investigated, although not in every case with the same care, bile salts (sodium taurocholate), amyl alcohol, ethyl alcohol, ether, chloroform, foreign serum, increase of temperature to 62°–64° C. The method mainly employed in addition to macroscopic and microscopic examination has been given in previous papers¹ and need not be repeated here. It consists in measuring the electrical conductivity of blood and of its serum, and in certain cases determining also the freezing point of the serum, after the various substances have been added to the blood. Since the normal erythrocytes are practically non-conductors as compared with the serum,² alterations in the condition of the corpuscles may be inferred from alterations in the conductivity of the blood. Exchange of water or dissolved substances between the corpuscles and the serum will also be reflected in alterations of the conductivity or freezing point of the serum.

For the experiments on hardened blood corpuscles I have employed chiefly sediments of formaldehyde-fixed blood carefully washed with salt solution to free it from serum constituents before the addition of the formaldehyde. Sometimes the entire blood has been used after fixing with formaldehyde. In a number of experiments Hayem's solution was used as the fixing agent.

Sapotoxin or Saponin.—I have previously shown³ that saponin markedly increases the permeability of the corpuscles for the electrolytes when it is added to formaldehyde blood, as shown by the great increase in the conductivity of the corpuscles. I have since found that a considerable increase can still be caused more than a year after the addition of the formaldehyde if the blood has not been allowed to dry. It is easy to show, by washing the corpuscles repeatedly with nine-tenths or one per cent NaCl solution, that the presence of the serum and the electrolytes

¹ Loc. cit.; also Jour. of Exper. Med., Vol. vi, p. 257.

² Cf. Centralb. f. Physiologie, 1897, xi, S. 332; Jour. of Physiol., 1899, xxiv, p. 356.

³ Jour of Exper. Med., vi, p. 257.

normally contained in it is not necessary for this result. It would seem even that NaCl and NH_4Cl pass with approximately equal ease through the formaldehyde-hardened corpuscles which have been acted on by saponin, and are not bound in the corpuscles to any extent. Unhardened blood-corpuscles or formaldehyde-hardened corpuscles not treated with saponin or sapotoxin permit NH_4Cl to pass much more readily into them than NaCl, and there is evidence that some of the NH_4Cl is bound by substances in the corpuscles or at any rate does not pass as freely out of them as it passes into them.

These conclusions are based upon experiments in which solutions of NH_4Cl and of NaCl of known and nearly equal conductivity are added in equal amounts to fresh blood, formaldehyde-hardened blood, and formaldehyde-hardened blood treated with saponin or sapotoxin. The resulting conductivities of the blood mixtures, their sera and their sediments are such that we must assume that in the case of the fresh and formaldehyde-hardened blood the NaCl does not appreciably pass into the corpuscle and become bound there. For this the conductivity of the sera of the mixtures is too high. Nor do the ions of the NaCl easily wander with their electrical charges into and out of the corpuscles, since for this the conductivity of the mixtures and their sediments is too low in comparison with the conductivity of the sera. Contrariwise, the conductivity of the mixtures, their sera and sediments are such that we must assume that in fresh and formaldehyde-hardened blood NH_4Cl is taken up by the corpuscles, since otherwise the conductivity of the sera would be too low. The ions of NH_4Cl must wander more easily through the corpuscles than those of NaCl, since the sediments have a higher conductivity in spite of the lower conductivity of the serum between the corpuscles.

After the formaldehyde-hardened corpuscles have been acted on by sapotoxin as has been said they seem to exhibit little if any power of binding either NH_4Cl or NaCl, since otherwise the observed conductivity of the serum would be too high; and the ions both of NaCl and NH_4Cl wander

easily through the corpuscles, since otherwise the conductivities of the mixtures and their sediments could not be so high in comparison with those of the sera. Some of these results are illustrated by Experiment I., others by experiments already published.

With the view of determining what constituents of the formaldehyde-hardened corpuscles are acted on by sapotoxin they were extracted with ether after thorough washing with nine-tenths per cent NaCl solution. The ether took up a considerable amount of cholesterin and also of a fatty-looking substance, probably lecithin. The entire ether extract was soluble in a mixture of chloroform and alcohol. The ether-extracted corpuscles were carefully washed many times with salt solution, and then suspended in a known volume of salt solution. For this suspension λ^1 was markedly greater than for a suspension of the unextracted formaldehyde corpuscles containing approximately the same proportions of corpuscles and salt solution. Sapotoxin caused no change in the λ of the suspension of extracted corpuscles. This renders it highly probable that sapotoxin increases the permeability of formaldehyde corpuscles by acting on the substances soluble in ether. If it does so after fixing by formaldehyde, we may assume without serious risk of error that it does so also in unfixed corpuscles. As a matter of fact it can be shown that when a solution of cholesterin is shaken up with sapotoxin so as to form a kind of emulsion, the sapotoxin is removed by the cholesterin, or rendered inert. If the proportions of sapotoxin and cholesterin have been properly chosen, the liquid obtained after filtering off the cholesterin has no laking power. Apparently a definite amount of cholesterin neutralizes a certain amount of sapotoxin. Formaldehyde corpuscles will also remove sapotoxin completely from a solution in twenty-four hours. The power of fresh dog's corpuscles to remove sapotoxin from a solution is markedly diminished or abolished by a temperature of 0° C. At this temperature blood was not laked in an hour by an amount of sapotoxin sufficient to cause almost instant laking

¹ This symbol will usually be employed for "conductivity."

at ordinary temperature. The sediment, separated by the centrifuge, did not at the end of this time contain enough sapotoxin to lake it at room temperature. The supernatant fluid caused rapid laking of the sediment or of a fresh specimen of blood at ordinary temperature.

During the process of extraction with ether the methemoglobin of the formaldehyde corpuscles is changed into hematin and hemochromogen. The hemochromogen bands become much stronger on addition of ammonium sulphide. Microscopically the corpuscles are well preserved, very distinct, smooth and round in outline, and they retain all their blood-pigment. On the average the corpuscles are somewhat smaller than in the original washed formaldehyde sediment. They preserve the normal dumb-bell shape when seen on edge. They are tinged more deeply with the blood-pigment than the unextracted corpuscles, partly, perhaps, because the latter are larger and the pigment therefore more diffuse, but perhaps also because the hemochromogen is a stronger pigment than the methemoglobin. There is well-marked clumping of the ether-extracted corpuscles, perhaps produced by changes wrought in the envelope during the action of the ether on it. Very numerous rouleaux are seen, the corpuscles being accurately apposed to each other as in fresh clotted blood.

Both the original washed formaldehyde corpuscles and the extracted corpuscles stain well with eosin. There seems a decided dearth of leucocytes in the ether-extracted specimen, although some may be seen whose nuclei do not stain very sharply with methylene blue, which tends to diffuse in the cytoplasm.

Amyl alcohol exerts much the same effect on the extracted corpuscles which it does on the unextracted formaldehyde corpuscles. The latter rise rapidly and completely to the top when a suspension of them is shaken up with amyl alcohol, a kind of emulsion being formed in which the corpuscles are entangled. The extracted corpuscles also rise, although not perhaps so readily or so completely. The same is true of corpuscles hardened with Hayem's solution.

Sapotoxin causes no change in the λ of corpuscles fixed by heat. The corpuscles were obtained by washing a sediment of dog's defibrinated blood free from serum and then gradually running a known amount of the washed sediment into nine-tenths per cent NaCl solution heated nearly to

¹ The question seems worthy of consideration whether the formation of rouleaux in clotting blood is not an agglutinative effect due to the action of auto-agglutinins.

boiling in the water bath. The corpuscles were fixed without laking. Under the microscope they were seen to be intact. A comparative experiment showed that the λ of a suspension of corpuscles was markedly increased by heat-fixing. The corpuscles are rendered equally permeable to NH_4Cl and NaCl , just as in the case of formaldehyde corpuscles which have been acted on by saponin or sapotoxin.

Sapotoxin also causes little or no change in the λ of corpuscles fixed by Hayem's solution (*i.e.*, by corrosive sublimate), and then washed repeatedly with salt solution till the washings gave practically no reaction for mercury on the addition of $(\text{NH}_4)_2\text{S}$. The want of effect of the sapotoxin is not due to any inhibitory action of the trace of HgCl_2 remaining in the extracorporeal liquid, since the addition of excess of the washings to sapotoxin solution does not prevent it from laking fresh blood in the normal way. Even for a short time after the addition of a one per cent solution of HgCl_2 in nine-tenths per cent NaCl solution to fresh blood sapotoxin produces laking. Also when a mixture of HgCl_2 solution and sapotoxin solution with enough NaCl to render the mixture isotonic is added to fresh blood laking occurs.

The corpuscles swell, though much more slowly than when blood is laked by sapotoxin in the absence of HgCl_2 . Often a body appears in the corpuscles during laking which looks like a nucleus, perhaps a portion of the hemoglobin shrunk together. Sometimes this is situated in the center of the corpuscles, sometimes it is eccentric. It often persisted as long as the ghost was watched. The ghosts did not become so indistinct at any time as in ordinary laking, and after a short time they began to grow more distinct. A curious point was observed when a drop of blood and a drop of the laking mixture were carefully covered by a slip so that a sharp interface separated them. The greatest number of laked corpuscles were to be found not at the very interface, but a little way further into the blood-drop. At the interface all the corpuscles first became crenated. In the region of maximum laking they were all still round, as in unlaked blood. The explanation perhaps is that the HgCl_2 had not reached the region of maximum laking, while the sapotoxin had. The HgCl_2 would preserve from rapid laking those corpuscles which it had reached. HgCl_2 is certainly a powerful fixative for the stroma. When it is added to water- or saponin-laked blood the ghosts, which before were

faint, instantly stand out with great distinctness. This strong fixing action on the stroma may be the reason why sapotoxin does not alter the λ of sublimate-hardened corpuscles. In this connection experiments are being made to determine whether ether extracts the same substances, and as much of them, from HgCl_2 corpuscles as from formaldehyde corpuscles.

That the negative result is not due to the sublimate-fixed corpuscles being so thoroughly hardened that they cannot be affected by any laking agent is shown by the fact that they are readily laked by being heated in distilled water to a temperature certainly not higher than that required for the heat-laking of fresh blood. I have not determined the lower limit of temperature at which laking of the sublimate corpuscles occurs, but have observed it to take place at as low as 54°C . The spectrum of the solution shows a band in the red (hematin). On adding a drop or two of $(\text{NH}_4)_2\text{S}$ the color changes from brown to reddish and the hemochromogen bands appear. On shaking up with air, two strong bands in the position of the oxyhemoglobin bands replace the hemochromogen spectrum. Then the oxyhemoglobin bands fade out, being succeeded by the band of reduced hemoglobin, and that by the hemochromogen bands. The same sequence was seen after addition of $(\text{NH}_4)_2\text{S}$ in excess to formaldehyde blood, or to fresh blood in which the hemoglobin had been changed to alkaline hematin by NaOH .¹ The ghosts of the sublimate corpuscles laked by heating in water are well preserved, and often aggregated in clumps. They disappear entirely, or become very faint, on the addition of $(\text{NH}_4)_2\text{S}$. The same thing happens when the unlaked sublimate corpuscles are acted on by $(\text{NH}_4)_2\text{S}$, which lakes them at once. The ghosts which have not been broken up are rendered more distinct by hydroxylamine hydrochlorate, which precipitates the free hemoglobin. Sachs² showed that substances which can combine with Hg will lake sublimate-fixed corpuscles. He employed potassium iodide and sodium hyposulphite. I can entirely confirm this result. I have used chiefly $(\text{NH}_4)_2\text{S}$

¹ Cf. Menzies. *Jour. of Physiol.*, Vol. xvii, pp. 402, 415.

² *Münch. med. Wochensch.*, February 4, 1902.

and H_2S . Ammonia causes the sublimate corpuscles to swell up and become indistinct. The H_2S makes them still more indistinct, some appearing to break up altogether. But if H_2S be added, then NH_3 , and then Löffler's methylene blue, it is seen that many have survived and are stained blue.

My results indicate that $(\text{NH}_4)_2\text{S}$ not only removes Hg from its combination with the blood-pigment, but also from its combination with the stroma.

The water-laking of sublimate corpuscles differs from that of normal corpuscles in that it does not take place at ordinary temperature. It agrees with normal water-laking in being prevented by the presence of an amount of salt in the water sufficient to render it hyperisotonic. A sublimate sediment suspended in ten per cent NaCl solution does not lake at all on being heated even to boiling. In addition to the osmotic effect of salt solutions there seems to be some other action exerted by NaCl, for when sublimate corpuscles have been in contact with a ten per cent NaCl solution for a little time, they will not lake on heating when enough water is added to reduce the strength to nine-tenths per cent, nor even when suspended in distilled water. The addition of ten per cent NaCl solution also prevents the laking action of $(\text{NH}_4)_2\text{S}$. Even if the sublimate corpuscles after the action of NaCl be separated and suspended in water, the addition of $(\text{NH}_4)_2\text{S}$ will not cause laking either in the cold or on heating.

Laking of formaldehyde blood-corpuscles.—None of the ordinary laking agents cause the liberation of the blood-pigment from well-fixed formaldehyde corpuscles. For example, water produces no such action even at the temperature of ordinary heat-laking. Nor does the heating of a suspension of formaldehyde corpuscles in nine-tenths per cent NaCl solution to the temperature of heat laking produce the least change in the λ . After the addition of ammonia, however, in very small amount formaldehyde corpuscles are capable of being laked. The color changes immediately on the addition of the ammonia from brown to red. The spec-

troscope shows that oxyhemoglobin has replaced methemoglobin in the corpuscles.¹ The addition of formaldehyde now causes the color again to become brown and the spectrum to change to that of methemoglobin. If more ammonia be added the color again becomes red. If a formaldehyde sediment be suspended in water and ammonia added, a relatively slow and partial laking occurs at the ordinary temperature. For a time the corpuscles retain all their blood-pigment, but are somewhat swollen. After standing for a night a considerable amount of blood pigment, in the form of oxyhemoglobin, will be found in solution in the water, but much will still remain in the corpuscles. The laking occurs rapidly and more completely at 60° C., and, no doubt, below that temperature. In this case, too, the blood-pigment in solution is oxyhemoglobin. Laking is also obtained at once on boiling, and no coagulation of the blood-pigment is seen. If the boiling be continued only for a few moments the pigment is still oxyhemoglobin. After more prolonged boiling hematin and hemochromogen are formed.

After water-ammonia laking the ghosts are large and swollen, and more distinct than ghosts obtained from unfixed blood. If the formaldehyde-fixing has gone a little farther, it is easy to get what seems to the eye to be laking by heating after the addition of ammonia, although little or none of the pigment may have come out of the swollen corpuscles. When they are filtered off it can be seen that they still contain all, or nearly all, the pigment. If the corpuscles be caused to shrink, as can be done by adding, *e.g.*, a solution of hydroxylamine hydrochlorate or of NH_4Cl , they resume with their normal size their normal tint.

The same change of color and of spectrum was produced by the addition of ammonia to bird's blood hardened by formaldehyde twelve months before, and to dog's blood hardened fifty-four weeks before. The corpuscles were well pre-

¹ For convenience I speak of the methemoglobin being changed into oxyhemoglobin on the addition of the alkali, since the chief bands of the spectrum are in the position of the oxyhemoglobin bands. (Cf. Araki, *Zeit. f. physiol. Chem.*, Bd. xiv, S. 405.) Some authors prefer to speak of the spectrum as that of alkaline methemoglobin. (Cf. Jäderholm, *Zeit. f. Biol.*, Bd. xx, S. 419.)

served. The excess of formaldehyde solution had not been removed from the blood, which was still moist. After the addition of ammonia the dog's corpuscles were observed to be somewhat swollen. No laking took place in either specimen on being suspended in water and heated to 65° C., nor on boiling (which instantly changed the color to brown, with formation of hematin), although the corpuscles looked paler than before in spite of the fact that no blood-pigment had escaped from them, and were certainly large in comparison with the original formaldehyde corpuscles, while still preserving their dumbbell shape when seen on edge. It is possible that ammonia liberates the hemoglobin in the interior of the corpuscle without producing that change in the envelope in this long hardened blood which would permit its escape. We have already found some evidence that sublimate fixes the stroma more readily than formaldehyde. Now ammonia does not cause laking of sublimate corpuscles, although it produces swelling of them. A very prolonged action of weak formaldehyde solutions (three or four per cent) such as were used in these experiments may, of course, fix the stroma much more firmly than a comparatively short action which is sufficient to fix the blood-pigment. Sodium hydrate does not nearly so easily cause laking of formaldehyde corpuscles as ammonia, although it readily changes the color to red, with the same change of spectrum as that given by ammonia.

Sapotoxin does not cause laking of washed formaldehyde corpuscles after the addition of just enough ammonia to change the methemoglobin into oxyhemoglobin, even when it is used in an amount far greater than is sufficient to cause immediate laking in fresh blood. There is no laking after many hours in the cold, and none on heating the mixture even to boiling. After treatment of ammonia-formaldehyde blood with sapotoxin for sixteen hours, the supernatant liquid was removed and the sediment suspended in water. On heating, it laked. The ghosts preserved the shape of normal corpuscles. Some of the saponin-ammonia-formaldehyde sediment suspended in nine-tenths per cent NaCl solu-

tion, and heated, refused to lake. The same is true of formaldehyde corpuscles suspended in nine-tenths per cent NaCl solution, then treated with sapotoxin, and then with ammonia, although the ammonia produces the usual red color.

It is a curious circumstance that the laking of ammonia-formaldehyde corpuscles, on heating, does not take place if they are suspended in nine-tenths per cent NaCl solution instead of water, but does take place if one per cent urea solution be substituted for the NaCl solution. This seems to indicate that urea easily penetrates the formaldehyde corpuscles after the addition of ammonia, while NaCl does not. The urea solution would in this case act much in the same way as water. It is well known that unhardened corpuscles are freely permeable to urea. But although NH_4Cl readily penetrates unfixed corpuscles, and penetrates formaldehyde corpuscles better than NaCl, at least for a considerable time after the formaldehyde has begun to act, ammonia-formaldehyde corpuscles are not laked on heating in one per cent NH_4Cl solution.

Washed formaldehyde corpuscles suspended in one per cent urea solution and heated are not laked, even on boiling. When boiled, an apparent precipitate forms. Microscopically it is seen that there is no precipitate whatever outside of the corpuscles, and the corpuscles do not seem to be altered. Nevertheless the appearance of a precipitate is probably due to some heat change in the corpuscles which renders them less transparent. The addition of ammonia while the suspension is still hot makes it much more, although not completely, transparent. The addition of ammonia after the suspension has cooled somewhat does not clear it up, but on boiling it clears up, although not so completely as when ammonia is added before boiling. Ammonia does not produce the red color when it is added to formaldehyde corpuscles which have been boiled. Many ghosts can be seen, most of which are more distinct than when urea solution and ammonia are both added to the formaldehyde corpuscles before boiling. In this latter case the ghosts are very faint and much swollen. That the results

obtained with urea solution were not due to any decomposition of the urea, with production of ammonia, during the heating, was shown by a separate experiment.

A combination of formaldehyde- and heat-fixing was obtained by suspending washed formaldehyde corpuscles in water and boiling. On the addition of ammonia and boiling it cleared up slightly, but not nearly so well as in the corresponding experiment with urea solution. The corpuscles were seen to be somewhat paler than before and somewhat swollen, but they preserved their normal shape. It is clear, therefore, that the urea alters the corpuscle in some way and that the urea solution does not act merely as water would.

We have seen that ammonia changes the methemoglobin of the formaldehyde corpuscles into oxyhemoglobin. The oxyhemoglobin can be changed back again into methemoglobin by washing the ammonia-formaldehyde corpuscles with water till the wash water has no alkaline reaction. To the eye the sediment now appears the same as before the addition of ammonia. It has the same brown color, and shows the same spectrum.

That it has been altered, however, is shown by the fact that sapotoxin has now little or no influence on the conductivity. Further, laking now takes place on heating the corpuscles in water, which is not the case with ordinary formaldehyde corpuscles till ammonia has been added. The addition of ammonia to the washed ammonia-formaldehyde corpuscles causes the usual red color and the spectrum again shows the oxyhemoglobin bands.

Formaldehyde corpuscles after extraction with ether are not changed in color by ammonia, nor is there any change in their spectrum nor any laking on heating, although some swelling of the corpuscles may take place, the normal shape being preserved. When heated with a little two per cent HCl some of the pigment goes into solution, and the spectrum shows the acid-hematin band in the red.

As has been said, some of the above results render it probable that formaldehyde does not fix precisely the same constituents of the corpuscle as the sublimate. It was there-

fore of interest to determine the effect of subjecting sublimate-hardened corpuscles to formaldehyde. It was found that the sublimate-formaldehyde corpuscles could not be laked by heating them in water even when all excess of formaldehyde was removed by repeated washing. The addition of formaldehyde accordingly abolishes this property of the sublimate corpuscles. It does not do so by expelling the mercury from the corpuscles, for the washings give no evidence of the presence of mercury. Mercury is still present in the corpuscles, for when they are treated with HCl and metallic copper a considerable film of mercury is deposited on the copper. That formaldehyde passes into the sublimate corpuscles is shown by the suspension forming into masses on the addition of the formaldehyde, as if the corpuscles swelled up. The color also changes, becoming a dark brown.

Bile Salts. — It is well known that bile salts are laking agents. I have shown (Experiment II.) that sodium taurocholate produces a similar action on formaldehyde-hardened corpuscles to that of sapotoxin, viz., an increase of their conductivity. In this case also the corpuscles appear to behave in the same way to NH_4Cl and NaCl .

There is this difference between the sodium taurocholate and crude saponin (or sapotoxin), that whereas in the laking of unhardened blood by the first-named reagent, at any rate in minimal dose, the conductivity is not increased beyond that of the control,¹ but may be diminished, laking by saponin causes an increase in the conductivity.

The Laking Action of Amyl Alcohol. — This is one of the most energetic of all the laking agents studied. The addition of it to blood in the proportion of one volume of amyl alcohol to seventy of the blood (Experiment III.) causes immediate and complete laking. Instead of an increase of λ , however, such as is caused by saponin, there is a diminution and a greater diminution than would be caused merely by the depressing influence on the conductivity of an

¹ By the "control" is meant in this connection a specimen of blood to which as much of a NaCl solution of approximately the same conductivity as that of the solution of the laking agent has been added as was added of the latter.

indifferent non-conductor added in equal amount. This is in favor of the view that in amyl alcohol laking the electrolytes of the corpuscles are not discharged along with the hemoglobin. It is also in favor of this that the ghosts (which may be found in great numbers), instead of being swollen and round as in saponin and water-laking, in both of which electrolytes pass out of the corpuscles, may be even smaller than the normal corpuscles, as is the case in heat and Na taurocholate-laking, in which the electrolytes do not appear to be so completely discharged from the corpuscles. Conductivity measurements, however, are less satisfactory for the study of laking in the case of substances like amyl alcohol, which do not mix with watery solutions, than in the case of the laking agents hitherto studied, and therefore I desire it to be understood that these statements are provisional. So far as Experiment III. shows, no increase in the permeability of formaldehyde-hardened corpuscles to electrolytes is produced by amyl alcohol. But amyl alcohol certainly affects the envelopes of such corpuscles, causing them to become "agglutinated," so that if a little of it is shaken up with a suspension of formaldehyde-hardened corpuscles in a watery liquid, a kind of jelly-like emulsion of the corpuscles and the amyl alcohol rises rapidly to the top, leaving the subjacent liquid free from corpuscles. Under the microscope the corpuscles may be seen to be aggregated together in groups. This action occurs even when the formaldehyde blood has stood nine months exposed to the air and is almost as hard as hard rubber. It occurs in the case of nucleated as well as non-nucleated corpuscles. It is difficult to make out any such "agglutinative" effect in the fresh blood, possibly because, as Flexner and Noguchi¹ have pointed out in connection with their important study of snake venom, rapid hemolysis obscures the agglutinative phenomena. So far as I have been able to make out, sapotoxin and Na taurocholate do not appear to have any such "agglutinative" effect either on fresh or formaldehyde-fixed corpuscles. Nor does amyl alcohol cause such a marked effect on washed

¹ Jour. of Exp. Med., 1902, Vol. vi, p. 277.

formaldehyde corpuscles as on formaldehyde blood with the serum elements present, although it carries up the corpuscles in the same way.

Water Laking. — Well-marked agglutination precedes the laking of unfixed blood by water. This can be easily studied when laking is caused by a watery solution of methylene blue. The corpuscles assume curious irregular shapes. Sometimes they become spindle-shaped and may be arranged in rows like a tissue with spindle cells. Although several corpuscles may sometimes seem to fuse together, no distinct outline being seen where they abut on each other, this is not really the case. When they lake the ghosts are always seen to be distinct, although they may be agglutinated too, and their outline is equally sharp all round. Before the blood has laked careful examination always discloses a faint indication of the boundaries between the individual corpuscles in a clump. The difference of refractive index is so slight that it does not seem as if any liquid can exist between the adjacent corpuscles. After the corpuscles touch each other it may be that all the intervening liquid passes into them. It may perhaps be the case that the intimate contact thus produced is an important factor in the agglutination. Rouleaux forms are exceedingly common in water laking even where the corpuscles have become elongated or spherical. The shape in this case is, therefore, not the determining factor in rouleaux formation. 'In laking of dog's corpuscles by Necturus serum, which is preceded by agglutination, it is also seen that while the corpuscles swell and become spherical before laking they need not do so before agglutinating. Crenated corpuscles can agglutinate and corpuscles which have preserved their normal shape are also seen assuming rouleau forms.

Ether Laking. — This is illustrated in Experiments IV. and V. In spite of the objection just mentioned to inferences drawn merely from measurements of conductivity in the case of such laking agents, two results seem to follow clearly from Experiment IV. (1) That when added gradually in just sufficient amount to cause complete liberation of the hemo-

globin ether does not cause any notable liberation of electrolytes from the corpuscles. (2) That in larger amount it brings both the hemoglobin and the electrolytes out of the corpuscles. The addition of one cubic centimeter of ether to five cubic centimeters of blood caused λ to diminish only from thirty and thirty-two one-hundredths to twenty-seven and forty-nine one-hundredths. In other words, when the blood mixture contained nearly seventeen per cent of its volume of the non-conducting ether its λ was diminished only a little over nine per cent. Now the addition of this amount of ether to a simple solution of electrolytes would have diminished λ at least four times as much.¹ A diminution of more than forty per cent in the original λ of the blood is actually caused in the same experiment by the gradual addition of six-tenths cubic centimeter of ether to five cubic centimeters of the blood, twelve per cent. of ether being present in the blood mixture, an amount apparently just sufficient to bring about complete laking. With the microscope in the blood laked by the addition of one cubic centimeter of ether at once no ghosts could be seen, but only granular masses agglutinated together, resembling in some respects leucocytes, although no nuclei were brought out by methylene blue. In the gradually laked blood many ghosts were seen on the addition of methylene blue. Possibly the ghosts are fixed by the gradual addition of the ether. In both specimens numerous hemoglobin crystals were present.

In Experiment V. ether was added to a suspension of corpuscles just sufficiently fixed by formaldehyde to prevent immediate water laking. A certain amount of laking was produced by water in excess after a contact of twenty-four hours. The object of the experiment was to see whether the permeability of the corpuscles is affected by ether as it is by saponin and Na taurocholate. While the experiment can only be considered a preliminary test, it does not afford any evidence that this is the case.

Relation of Water Laking to Other Forms of Laking:—

¹ See Ostwald's *Allgemeine Chemie*, Bd. ii, for the influence of non-conductors on the conductivity of solutions of electrolytes.

The fact that certain of the laking agents increase the permeability of formaldehyde-hardened corpuscles to electrolytes suggests that in laking of the unhardened blood their primary action may be an increase of permeability to the electrolytes or other dissolved substances of the serum, or of any watery solution in which the corpuscles are suspended. Since dissolved substances which freely penetrate a membrane exert no osmotic pressure, the action of such laking agents would come practically to the same thing as the addition of water to the blood. Nolf and Hédon have put forward the idea that saponin laking is really a water laking. I stated in my last paper ¹ that my results on saponin action were compatible with this theory, but was careful not to go further. Further observations have convinced me that the theory is not tenable for any of the laking agents investigated by me, with the possible exception of foreign serum.

Evidence on this point may be obtained by adding to the laking agent or to the blood substances which do not penetrate the normal corpuscles, *e.g.*, cane sugar, in such amounts that even if the laking agent rendered the corpuscles freely permeable to the electrolytes of the serum, the serum would still remain isotonic or hypertonic to the corpuscles. It may be objected that laking agents may increase the general permeability of the corpuscles not only for the salts of the serum, but for other substances, in which case the addition of these substances would not prevent water laking. This difficulty may be overcome by testing the effect of laking agents on blood sediments separated by long centrifugalization from the greater part of the serum. I have obtained from defibrinated blood sediments only seven or eight per cent of whose volume consisted of serum, as determined by the electrical method.² Since the formula used for this determination is, of course, most accurate when the proportion of corpuscles to serum is about that of ordinary defibrinated blood and less accurate for extreme variations from the normal, the calculated result was checked by actual measurement of the

¹ Jour. of Exp. Med., loc. cit.

² Jour. of Physiol., xxiv, p. 356.

amount of serum removed by the centrifuge and deduction of this amount from the serum in the defibrinated blood as determined by the electrical method. One can, of course, combine the addition of substances not likely to penetrate the corpuscles even after the action of the laking agent with the getting rid of as much water as possible by centrifugalization, and washed sediment may be used from which all the serum constituents have been separated.

It has been shown in Experiment VI. that dog's defibrinated blood to which cane sugar has been added is laked by heating at the usual temperature of heat-laking, although not so rapidly as blood to which no cane sugar has been added.

In Experiment VII. it is shown that heat laking occurs in blood to which several volumes of approximately isotonic cane sugar solution have been added.

Experiment VIII. illustrates the fact that saturation of blood with cane sugar does not prevent nor delay laking by heat or sapotoxin, although a small number of the corpuscles seem to resist the laking action better in the presence of cane sugar. Laking by sodium taurocholate, although not prevented, seems to take place less rapidly when sugar is present. Had the delay occurred in case of the sapotoxin laking it might possibly have lent some support to the water-laking theory, since sapotoxin undoubtedly causes the corpuscles to swell up and become spherical before they discharge their hemoglobin. This it does even when it lakes blood saturated with cane sugar, and it is curious to note how suddenly the intensely crenated corpuscles become round and of smooth outline and then immediately fade out. Sodium taurocholate, however, possesses the property of being able to lake the corpuscles without causing them to swell up and become spherical. The diminution in the rapidity of the laking action of the taurocholate which is produced by cane sugar would, therefore, appear to be due to some other cause than interference with the passage of water into the corpuscles.

In Experiment IX. the influence of the addition of cane sugar on the laking action of foreign serum is illustrated.

Even when dog's serum is saturated with sugar it causes laking of rabbit's blood corpuscles, although the laking is not so complete nor so rapid as in absence of the sugar.

In Experiment X. the conductivity of rabbit's blood which has been acted on by dog's serum containing various amounts of cane sugar is compared with that of the same blood to which equal quantities of the sugar-serum whose laking property has been destroyed by heat have been added. I showed before¹ that foreign serum, unlike more violent laking agents, such as saponin, does not cause an increase in the conductivity of the blood when laking occurs, apparently because it permits the electrolytes of the corpuscles to remain in the ghosts. In general, indeed, the conductivity of the laked blood is somewhat less than that of blood to which heated serum has been added, notwithstanding the fact that there is no difference in the conductivity of heated and unheated serum. Experiment X. shows that when rabbit's blood, seventeen hours after being drawn, is acted on by dog's serum containing sugar the same relation as regards the conductivity is preserved.

The same thing is shown in Experiment XI. for stale and in Experiment XII. for perfectly fresh rabbit's blood.

In Experiment XII. dog's serum which had partially lost its laking power by standing, four days having elapsed since the dog's blood was drawn, was employed in order to see whether when the resistance of the corpuscles was at its maximum and the toxic power of the serum small the sugar might not exert a more marked restraining influence. The dog's serum was the same as that used three days earlier in Experiment X. The outcome of all these observations on foreign serum is that cane sugar, although it certainly diminishes the laking power, does not abolish it nor alter its type.

Experiment XIII. shows that alcohol laking also is not prevented by cane sugar.

In Experiment XIV. the influence of excess of ten per cent NaCl solution on heat laking was investigated, with the result that laking was found to occur at the usual tempera-

¹ Jour. of Phys., xxiv, p. 211.

ture, although it was perhaps not complete. It also occurred in a sediment of corpuscles washed nearly free from serum and then suspended in saturated NaCl solution.

In Experiment XV. it is shown that complete laking is produced by saponin, sodium taurocholate (both added in substance), alcohol, amyl alcohol, ether, chloroform, and heating to 62–64° C. in a sediment of dog's corpuscles containing no more than seven to eight per cent of its volume of serum; and in the case of the observations on the sediment after partial desiccation considerably less than this amount. The addition of three times as much water as is present in the serum between the corpuscles causes only very incomplete laking.

From all these observations the conclusion seems to follow that although some of the laking agents investigated (crude saponin, sapotoxin, sodium taurocholate) do increase the permeability of the formaldehyde-fixed corpuscles, and therefore presumably of the unhardened corpuscles, to certain electrolytes and possibly to other dissolved substances in the serum, and thus disturb the osmotic equilibrium between serum and corpuscles, the laking produced by them is not primarily a water laking. In the case of some laking agents, foreign serum and heat, for instance, no increase of permeability of the formaldehyde-fixed corpuscles, or a very small one, seems to take place.

Thus in Experiment XVI. a quantity of dog's serum which was known to be more than enough to produce laking in unhardened rabbit's blood was added to the same blood after sufficient hardening in formaldehyde solution to prevent laking. The serum constituents and the formaldehyde which had not entered the corpuscles were removed by washing with NaCl solution and centrifugalization, and the corpuscles were suspended in NaCl solution. The conductivity of the hardened blood mixture after the addition of the dog's serum was a little greater than that of the corresponding mixture to which heated dog's serum had been added, and the same was true of the sediments separated from the mixture. A slight increase in the permeability of the corpuscles may, therefore,

have been produced by the foreign serum, but much less than is produced by sapotoxin or sodium taurocholate.

In Experiment XVII. heating to the temperature of heat laking produces no change whatever, and in Experiment XVIII. only a very slight change in the conductivity of suspensions of formaldehyde-hardened corpuscles, and therefore presumably no change in their permeability. That this is not due to any "over-hardening" of the corpuscles is shown (in Experiment XVIII.) by the fact that saponin produces the usual increase of conductivity.

Leucolysis. — In connection with the work on laking of the red corpuscles, I have, of course, had numerous opportunities of noting the behavior of the leucocytes to the various laking agents employed. I do not propose at present to enter into the matter in any detail, but shall mention a few of the facts observed. It is easy to show that there are great differences in the leucolytic power of the laking agents, and that there is no obvious relation between the intensity of the erythrolytic and that of the leucolytic power of a given agent. A substance which possesses a powerful action on the red corpuscles may be comparatively innocuous to the leucocytes, and of two substances the one with the smaller erythrolytic action may be the more powerful leucolytic agent. Although some of these chemical and physical laking agents do exert a marked influence on both kinds of corpuscles, there may, perhaps, be some which are only erythrolytic, and others which are only leucolytic, just as in a biological laking agent, snake venom, according to Flexner and Noguchi,¹ the "dissolving principle for leucocytes is distinct from that for red cells."

Sapotoxin is a more energetic laking agent for red corpuscles than sodium taurocholate. But in the doses which suffice to cause complete erythrolysis the bile salt is much the more powerful leucolytic agent. A very instructive comparison is afforded by observing under the microscope the action of the two substances on a drop of blood separated by a sharp interface from the solution of the laking agent.

¹ Loc. cit.

In one experiment dog's defibrinated blood, drawn thirty minutes before the observations were commenced, was subjected under the microscope to the action respectively of a two per cent solution of sodium taurocholate and a two per cent solution of sapotoxin. In both cases the laking agent was dissolved in nine-tenths per cent NaCl solution. In both solutions the red corpuscles may be seen suddenly disappearing here and there along the line of contact. The abruptness with which a corpuscle disappears from among its neighbors, and the random manner in which the hemolytic agent seems to pick out the corpuscles which it decolorizes suggest the description of a battle, with men dropping here and there, while others near them remain unhurt, and those apparently the most exposed not necessarily falling first. If a leucocyte happens to emerge among the fading red corpuscles at the interface, it may hold out, in the case of the taurocholate, till all or most of the erythrocytes in front of it, *i.e.*, between it and the advancing solution, and some of those immediately in its rear, have disappeared, but intact leucocytes cannot be seen far out in the solution. In sapotoxin laking, on the other hand, numerous leucocytes may be observed far in advance of the line of unaltered red corpuscles, standing isolated in the field where they alone have survived. After a time some of the leucocytes succumb to the sapotoxin too, but there is a marked difference between the action of the two laking substances in the rapidity and completeness of the process. The progress of the leucolysis was also very well observed after staining the nuclei of the leucocytes of the fresh blood with a solution of methylene blue in salt solution. The lymphocytes were seen to persist longer in the taurocholate solution than the larger varieties of leucocytes, but eventually they disappeared too. The leucocytes with polymorphous nuclei, and the larger leucocytes in general, succumbed earlier.¹ In sodium taurocholate leucolysis it was seen that the nuclei persist for some time after the rest of the cell has become indistinct, but eventually they seem to break up too. If the leucocytes have been previously stained with methylene blue the place of the nucleus, as leucolysis goes on, may be seen to become occupied with diffuse blue granules, which persist for a little time and then also disappear.

Although sapotoxin does not cause the disappearance of the leucocytes to nearly the same extent as the bile salt, some may be observed to become altered and break up. A not uncommon change is the fading away (solution?) of the granules in the protoplasm of the polymorphous leucocytes, so that at a certain stage nothing is visible except the peripheral ring-like contour of the leucocyte and the nuclei inside it. Changes of shape are also caused and portions of the protoplasm of the leucocyte

¹ After making this observation I was interested to find in the paper of Flexner and Noguchi already referred to, the statement that the lymphocytes are more resistant to snake venom than the other varieties of white cells. The study of the action of various leucolytic agents on the granules of the different kinds of leucocytes would seem calculated to throw light on their nature, origin, and relationships.

sometimes extruded, so that what appear to be two or three small leucocytes may occupy the place of a single large leucocyte. This appearance does not seem to be due to the extrusion of the swollen lobes of the nucleus, although swelling of the nucleus takes place. Just as in the case of the taurocholate leucolysis the lymphocytes appear to be less affected by sapotoxin than the larger varieties of leucocytes. The addition of sodium taurocholate to blood already acted on by sapotoxin destroys the leucocytes spared by the sapotoxin, except a few of the smaller leucocytes.

It was incidentally observed in these experiments that the colored corpuscles when acted on by the taurocholate maintained their dumbbell shape, and although often somewhat distorted did not swell up and become spherical before disappearing. In sapotoxin laking, on the other hand, although up to the very moment of laking the form of a corpuscle might have been normal, it all at once became spherical and instantly disappeared. When laking by the taurocholate is caused to proceed very slowly, as in blood well diluted with nine-tenths per cent NaCl solution, some of the red corpuscles may be seen to swell up and become spherical before the hemoglobin is discharged.

After laking by each substance the ghosts of the red corpuscles could be seen when carefully looked for, especially by the aid of methylene blue or iodine.

The addition of water to dog's blood causes distinct swelling of the leucocytes. Some swelling is also caused by watery solutions of ammonium chloride, whose action on the red corpuscles is, as we have seen, essentially water-laking. Apart from the swelling the leucocytes are not so profoundly altered as by the leucolytic agents hitherto described. The nuclei stain, apparently in a normal manner, with methylene blue, and granules have been seen (in blood laked by ammonium chloride solution) in the protoplasm. The addition of taurocholate solution to blood laked by water or by NH_4Cl solution causes the disappearance of the leucocytes, the larger varieties disappearing first and the smaller later on, just as in normal blood. Sapotoxin has the same effect as sodium taurocholate on the leucocytes of water-laked blood.

In one experiment a watery solution of methylene blue was added to water-laked dog's blood. The nuclei of the leucocytes were well stained. Then taurocholate solution was added. A large mononuclear leucocyte which showed no granules in its protoplasm was watched. Its nucleus

immediately lost its stain, which seemed to diffuse in the cell-substance, the latter becoming blue, although colorless before. No granules appeared in the cell substance. The outline of the nucleus was circular and remained sharp. Ultimately the leucocyte disappeared. The lymphocytes resisted longer, but on mixing the taurocholate solution more thoroughly with the laked blood, all the leucocytes speedily lost the stain of their nuclei and disappeared. Some of the large leucocytes were seen to break up suddenly, leaving a kind of sinuous thread or fiber, apparently corresponding in length to the perimeter of the optical section of the leucocyte. The swollen round water-laked red corpuscles became smaller, and most of them seemed to break up also. This was also the case on the addition of sapotoxin to the water-laked blood.

Amyl alcohol, although it laves blood so energetically, does not appear to possess a high degree of leucolytic power. At any rate, numerous leucocytes, whose nuclei stain well with methylene blue, may be seen in dog's blood after complete laving with this agent. The ghosts of the red corpuscles do not appear to be swollen as in water laving, but, if anything, smaller than the intact corpuscles. Necturus serum, which laves the red corpuscles of dog's blood, causes the nuclei of the polymorphous leucocytes to become more distinct.

Summary.

1. Ether dissolves out a considerable amount of material (including cholesterin) from washed formaldehyde-fixed corpuscles. Sapotoxin produces no change in the conductivity of a suspension of such ether-extracted corpuscles. It probably produces the increase of permeability in the unextracted formaldehyde corpuscles by an action on substances soluble in ether. It is to be presumed that it also acts in this way when it laves unfixed corpuscles.
2. Sapotoxin causes no change in the conductivity of a suspension of washed sublimate-fixed corpuscles.
3. Sublimate-fixed corpuscles can be laved by heating them in water.
4. The addition of ammonia to formaldehyde-fixed corpuscles changes the methemoglobin spectrum into that of oxyhemoglobin (alkaline methemoglobin). The corpuscles

can then be laked by the addition of water, slowly in the cold, rapidly on heating.

5. Corrosive sublimate and formaldehyde do not seem to fix precisely the same constituents of the corpuscles. The former fixes the stroma very rapidly. The mercury can be withdrawn from its combination with the stroma by H_2S . On the further addition of NH_3 or on adding ammonium sulphide at once, the ghosts may disappear.

6. Amyl alcohol in its laking action does not appear to cause the liberation of the electrolytes of the corpuscles.

7. Ether in an amount just sufficient to cause laking does not seem to liberate the electrolytes of the corpuscles, but in larger amount it seems to do so and to break up the ghosts.

8. Some of the agents investigated are active leucolytic agents (especially sodium taurocholate), but there is no direct proportion between their erythrolytic and their leucolytic power.

9. Although some of the laking agents investigated (crude saponin, sapotoxin, sodium taurocholate) increase the permeability of formaldehyde-hardened corpuscles, and might, therefore, be supposed to produce their laking action on unhardened corpuscles by increasing their permeability to the dissolved substances of the serum and thus causing water-laking, evidence is brought forward that this is not the primary action of these substances, although it may be a secondary one. For

(1) Laking is produced by all the agents investigated (heat, sapotoxin, sodium taurocholate, foreign serum) in blood to which excess of cane-sugar, which does not penetrate the normal corpuscles, has been added. Even when blood is saturated with cane-sugar laking occurs. Only in the case of foreign serum is the laking notably delayed and rendered incomplete by the presence of the sugar. Heat-laking is not prevented by sodium chloride.

(2) Laking is also produced (by saponin, sodium taurocholate, both added in substance, ethyl alcohol, amyl alcohol, ether, chloroform, and heating to 62° – 64° C.) in

sediments of corpuscles freed as far as possible from serum and containing so little inter-corpuscular water that even the entrance of the whole of it into the corpuscles could only cause a small amount of water-laking. For example, laking was normal in a sediment containing seven to eight per cent of serum, and even less.

Addendum. — Since the above was written I have made observations on the blood of *Necturus*, which is especially suitable for the microscopical study of hemolysis on account of the great size of the corpuscles, both colored and colorless. I expect shortly to publish a paper on the laking of nucleated colored corpuscles, but embrace this opportunity of making a brief preliminary report on the effect of certain laking agents on *Necturus* blood.

1. The hemoglobin of *Necturus* crystallizes with great readiness when the blood is laked.
2. Several of the laking agents investigated cause exquisite intraglobular crystallization, especially Na taurocholate, and after it sapotoxin-water and watery solutions of substances like NH_4Cl , which easily penetrate the corpuscles. In crystallization within the corpuscles portions of the hemoglobin (or hemoglobin containing stroma) first become differentiated from each other, usually as round or slightly oval bodies with faint outlines. The outlines rapidly grow more distinct and more angular, the portions of intra-corpuscular hemoglobin being bodily transformed into crystals of corresponding size, enclosed in the envelope of the corpuscle, which can be seen passing over the gaps between adjacent crystals.
3. Sodium taurocholate after a time causes great swelling of the nuclei of the colored corpuscles, the nucleus coming to fill up nearly the whole of the ghost. The corpuscle does not become globular, as is the case in water-laking, but preserves its oval form. Sapotoxin does not cause swelling of the nuclei.
4. Heat-laking takes place at a temperature several degrees below that necessary for laking of dog's corpuscles.
5. Ammonia causes marked swelling of the nuclei of colored corpuscles which have been fixed by Hayem's solution, the whole corpuscle becoming very indistinct. $(\text{NH}_4)_2\text{S}$ has a similar effect. H_2S causes the corpuscles to become indistinct without affecting the nuclei, which are seen to be tinged with the blood-pigment.
6. By treating sublimate-fixed colored corpuscles first with H_2S , then with NH_3 , and then with Löffler's methylene blue, which causes most of the swollen corpuscles and nuclei to shrink, a membrane surrounding the corpuscle may be demonstrated. A membrane surrounding the nucleus can also be made out.

7. Formaldehyde-fixed colored corpuscles suspended in water swell and become pale on being heated to 60°C after addition of NH_3 , and this even when the blood-pigment still remains in them. The nuclei are not swollen. Solutions which cause the corpuscles to shrink, *e.g.*, hydroxylamine hydrochlorate, bring back the normal tint. Löffler's methylene blue causes shrinking and stains deeply both the nucleus and the rest of the corpuscle.

8. Water causes leucolysis after a distinct period of resistance. The large leucocytes swell greatly, some of them becoming larger than the colored corpuscles. Their nuclei also swell, and many of the coarse granules in the protoplasm seem to become swollen vesicles. Some of the granules may dissolve; many of the coarse granules escape from the leucocytes. Sapotoxin causes the disappearance of most of the leucocytes, the large coarsely granular ones being left. Na taurocholate does not seem to have such a pronounced leucolytic action as it has on mammalian leucocytes. It causes Necturus' leucocytes to become dim, although they seem to swell little, if at all.

EXPERIMENT I. — The laking power of a sample of sapotoxin was first determined on dog's defibrinated fresh blood. A two per cent solution of sapotoxin in nine-tenths per cent NaCl was made. Four-tenths cubic centimeters of this added to five cubic centimeters of the dog's blood caused rapid and complete laking; three-tenths cubic centimeters, partial laking; two-tenths cubic centimeters, no effect, except some darkening after several hours.

Time.		λ^1
11.30 A.M.	A sediment of dog's formaldehyde blood washed in one per cent NaCl and centrif. Then mixed with one per cent NaCl = A.	47.29
11.32 "	To eleven cubic centimeters of A add eight-tenths cubic centimeters of two per cent sapotoxin = A + sapotoxin.	
11.55 "	82.57
11.50 "	Five cubic centimeters of A + thirty-six one-hundredths cubic centimeters of the NaCl. Used in making the sapotoxin solution (control).	
3.15 P.M.	48.29
11.42 A.M.	Five cubic centimeters of A + five cubic centimeters NH_4Cl solution.	
2.39 P.M.	71.61
	Top ²	93.89
	Bottom ²	45.27
11.42 A.M.	Five cubic centimeters of A + five cubic centimeters NaCl solution.	
2.33 P.M.	78.97
	Top ²	109.74
	Bottom ²	31.81
11.59 A.M.	Five cubic centimeters of A + sapotoxin + five cubic centimeters NH_4Cl solution.	
3.00 P.M.	94.94
	Top ²	101.00
	Bottom ²	87.72
11.59 A.M.	Five cubic centimeters of A + sapotoxin + five cubic centimeters NaCl solution.	
2.47 P.M.	101.40
	Top ²	110.70
	Bottom ²	89.58
	The NH_4Cl solution	112.17
	The NaCl solution	120.14
	The same NaCl solution was used for making the sapotoxin solution.	

¹ As in all the tables, λ is an abbreviation for $\lambda (5^\circ) \times 10^6$, the conductivity (at 5°C.) being expressed in reciprocal ohms.

² Separated by centrifuge twenty-four hours after mixture.

EXPERIMENT II.

Time.		λ
March 19.	First determined the laking power of sodium taurocholate on dog's defibrinated blood.	
8 A.M.	To portions of two cubic centimeters of dog's defibrinated blood (after removal of a part of the serum) added respectively, one-tenth cubic centimeter, two-tenths cubic centimeter, three-tenths cubic centimeter, and eight-tenths cubic centimeter of a two per cent solution of sodium taurocholate (Schuchardt) in NaCl solution. Complete laking observed only in the last. It was distinctly laked in one and one-half hours (might have been earlier). The portion to which four-tenths cubic centimeter was added was somewhat darkened, and twenty-four hours later was seen to be almost completely laked.....	λ
	The sodium taurocholate solution.....	129.34
	The NaCl solution used in making it.	119.02
	To a sediment of formaldehyde blood practically free from serum added one-fourth its volume of NaCl solution. Call the mixture B.	31.81
	Top of B.....	98.26
3.25 P.M.	Ten cubic centimeters B + four cubic centimeters of the sodium taurocholate solution.	
4.09 "	74.78
	(Nineteen hours later).....	73.69
	Top (a few corpuscles).....	104.32
3.55 "	To ten cubic centimeters of B added four cubic centimeters of NaCl solution (control).	
4.16 "	51.42
4.30 "	Added four one-hundredths cubic centimeters of ten per cent NaCl to the control.	
4.52 "	52.38
	(Nineteen hours later).....	51.95
	Top (a few corpuscles).....	103.89
	Added to four cubic centimeters of the NaCl solution four one-hundredths cubic centimeters of ten per cent NaCl solution.....	128.03
5.07 "	Added to five cubic centimeters of defibrinated blood two cubic centimeters of sodium taurocholate solution. Begins to darken almost at once, though not laked in fifteen minutes. Much darkened in twenty minutes.	
	(After sixteen hours) completely laked	54.99

EXPERIMENT II. — *Continued.*

Time.		A
5.07 P.M.	Added to five cubic centimeters of defibrinated blood, two cubic centimeters of the NaCl solution + two one-hundredths cubic centimeters of ten per cent NaCl (control). (After sixteen hours) The defibrinated blood.....	59.37 38.82
March 21	A NH_4Cl solution	120.14
	A NaCl solution.....	119.02
3.25 P.M.	To the sediment of B + sodium taurocholate added half its volume of about seven per cent cane sugar solution. Call the mixture B'	44.79
3.30 "	To five cubic centimeters of B' added five cubic centimeters of the NaCl solution. (After sixteen hours) (Fifty-one hours later) Top (transparent, contains some hemoglobin) ..	77.76 78.97 90.54
3.30 "	To five cubic centimeters of B' added five cubic centimeters of the NH_4Cl solution. (After sixteen hours) (Fifty-one hours later)..... Top (somewhat turbid; though the corpuscles have settled)	73.27 72.84 84.78
4.40 "	To sediment from control added about half its volume of the seven per cent cane sugar solution. Call mixture B''.....	33.14
4.55 "	To five cubic centimeters of B'' added five cubic centimeters of the NaCl solution. (After fifteen hours)..... (Fifty-one hours later)..... Top	68.14 68.33 91.20
4.55 "	To five cubic centimeters of B'' added five cubic centimeters of the NH_4Cl solution. (After fifteen hours)..... (Fifty-one hours later)..... Top	63.85 62.59 83.94

EXPERIMENT III. — Seventy hours before the beginning of the observations defibrinated blood was obtained from a dog. To some of it two-thirds of its volume of a four per cent solution of formaldehyde in eight-tenths per cent NaCl solution was added, and the mixture, as well as the original defibrinated blood, left in the cold.

Time.		λ
12.15 P.M.	Added to the formaldehyde blood its own volume of one per cent NaCl.....	78.73
2.14 "	To five cubic centimeters of the mixture added seven one-hundredths cubic centimeter of amyl alcohol.....	77.52
2.37 "	77.29
3.07 "	
3.09 "	Added seven one-hundredths cubic centimeter more of the amyl alcohol.....	77.76
3.24 "	The formaldehyde solution (in NaCl solution).....	83.39
	To five cubic centimeters of the formaldehyde solution added seven one-hundredths cubic centimeter of amyl alcohol.....	78.73
12.20 "	To five cubic centimeters of the defibrinated blood added five cubic centimeters of one per cent NaCl.....	76.36
12.30 "	
12.45 "	To five cubic centimeters of this mixture added seven one-hundredths cubic centimeter amyl alcohol (laked completely; a few intact corpuscles can be seen; very numerous ghosts revealed by methylene blue).....	64.67
2.23 "	

EXPERIMENT IV.

Time.		λ
2.15 P.M.	Dog's defibrinated blood.....	30.32
	To five cubic centimeters blood added one cubic centimeter ether (lakes at once).....	
2.50 "	27.49
2.15 "	To five cubic centimeters blood added two-tenths cubic centimeter ether.....	
2.35 "	(Not laked).....	24.21
	Added one-tenth cubic centimeter more ether.....	
2.45 "	(Darkened, but not laked).....	21.93
3.45 "	Added one-tenth cubic centimeter more ether.....	
3.50 "	20.37
3.55 "	Added one-tenth cubic centimeter more ether (not yet laked, although very dark).....	
3.57 "	Added one-tenth cubic centimeter more ether (laked suddenly).....	18.00
4.01 "	
2 15 "	To five cubic centimeters blood added eight one-hundredths cubic centimeter ether.....	
3 20 "	(Not laked).....	29.44

EXPERIMENT V. — Twenty-four hours before the beginning of the observations dog's defibrinated blood was mixed with two-thirds of its volume of a four per cent solution of formaldehyde in eight-tenths per cent NaCl solution. Separated by the centrifuge, washed with water, and again centrifugalized. The sediment was now suspended in water.

Time.		λ
3.30 P.M.	The sediment suspended in water (no laking).....	3.69
3.34 "	To three cubic centimeters of this suspension added one cubic centimeter of ether (no laking).....	2.15
4.33 "	2.15

EXPERIMENT VI. — To dog's defibrinated blood drawn nineteen hours before and kept in the cold, cane sugar in substance was added. The blood was then heated to 64° C. for twenty minutes. It became laky. Under the microscope, a fair number of intact corpuscles could be seen. Heated for ten minutes longer. Is now completely laked and no intact red corpuscles can be found. The ghosts adhere closely in masses. The leucocytes do not appear to be affected.

A specimen of the same defibrinated blood was heated in the same water-bath simultaneously with the blood to which sugar had been added. In ten minutes the laking was complete.

EXPERIMENT VII. — To two cubic centimeters of dog's defibrinated blood added ten cubic centimeters of a twelve per cent solution of cane sugar (which is nearly isotonic with blood serum). Heated to 64° C. It is well laked. Microscopically, it is seen that most of the corpuscles have disappeared.

EXPERIMENT VIII. — To dog's defibrinated blood which had stood for three and one-half days in the cold, but in which the corpuscles were intact and which had no putrefactive odor, more than enough cane sugar to saturate it was added. A considerable quantity remained undissolved.

Heated some of the sugar-containing blood to 64°. It darkens and lakes as soon as a specimen of the same blood without sugar, kept in the bath at the same time. A few intact red corpuscles can be seen in both, but they are more numerous in the sugar-blood. The sugar-containing blood is somewhat darker than the other in reflected light. Three hours later this difference is still present. Some intact corpuscles can still be seen in the sugar-blood, but none in the other.

To five cubic centimeters of the dog's blood saturated with sugar added four-tenths cubic centimeters of a two per cent solution of sapotoxin in nine-tenths per cent NaCl solution, and the same amount to five cubic centimeters of the blood without sugar. The two specimens darken and

lake with equal rapidity. Microscopically some intact corpuscles can be seen in the sugar-blood, few or none in the other. This is the case also three hours later.

To five cubic centimeters of the dog's blood saturated with sugar added two cubic centimeters of two per cent solution of sodium taurocholate in nine-tenths per cent NaCl solution, and the same amount of the taurocholate solution to five cubic centimeters of the normal blood. Both specimens begin to darken in a few minutes. In an hour the one without the sugar is markedly darker than the other, and better laked, although there is some laking in the sugar specimen too, which, however, contains numerous intact corpuscles, crenated and otherwise distorted. Added one cubic centimeter more of the taurocholate solution to each, and also to the sugar specimen an additional amount of sugar sufficient to keep it saturated, some remaining undissolved. In a few minutes the sugar specimen is markedly darker than before, and an hour later comparatively few intact corpuscles remain. In the specimen without sugar there are practically no intact corpuscles. In both leucocytes can be seen. In the specimen without sugar some hemoglobin crystals are found, but none in the other. On allowing the laked blood to dry somewhat on a slide numerous hemoglobin crystals form in all the specimens without sugar, but not in the sugar specimens. The presence of sugar seems to hinder the crystallization of the hemoglobin.

EXPERIMENT IX.

Time.		λ .
March 7. 11.35 A.M.	A sediment of rabbit's blood (obtained by sedimentation). About half the total volume of the defibrinated blood was pipetted off as clear serum. The serum of the rabbit's blood.	23.85 82.30
	The sediment is not laked by forty per cent formaldehyde, added drop by drop.	
March 8. 12.04 P.M.	To one cubic centimeter of the sediment of rabbit's blood added five cubic centimeters dog's serum (from blood drawn March 6).	
1.40 "	Is entirely laked.	
2.45 "	65.00
2.35 "	To one cubic centimeter rabbit's sediment added five cubic centimeters dog's serum, heated ten minutes to 57-64° C. Sediment of corpuscles settled in a few minutes.	
3.14 "	67.42
12.04 "	To one cubic centimeter of rabbit's sediment added five cubic centimeters of the same dog's serum saturated with cane sugar.	
1.40 "	Not laked completely. Very distinctly darkened. Microscopically, numerous ghosts and also many intact corpuscles seen.	
2.53 "	26.66
	After twenty hours, some sediment in the tube, but most of the Hb is in solution in the serum.	
2.00 "	The rabbit's sediment	21.28
2.08 "	The dog's serum with sugar (probably not quite saturated)	
2.19 "	The dog's serum	25.74
2.35 "	To one cubic centimeter rabbit's sediment added five cubic centimeters dog's serum saturated with sugar at room temperature and heated to 57-64° C. for ten minutes.	79.97
3.20 "	Corpuscles do not sink at all readily	26.91

EXPERIMENT X. — Obtained rabbit's defibrinated blood by decapitation seventeen hours before the beginning of the observations. Kept in the cold. For the rabbit's blood $\lambda = 43.71$; for the rabbit's serum $\lambda = 77.29$. Percentage of serum in the rabbit's blood 73.6. Obtained serum from the clot of dog's blood, drawn forty-three hours before the beginning of the observations. For the dog's serum $\lambda = 82.04$; for the dog's blood $\lambda = 41.22$. Percentage of serum in the dog's blood 66.6. For some of the dog's serum which was heated for fifteen minutes at 62°-64° C. $\lambda = 82.04$. For a mixture of equal volumes of the dog's serum and 10 per cent cane sugar solution $\lambda = 40.05$. For the 10 per cent cane sugar solution λ is less than 0.2.

Time.		λ.
9.40 A.M.	To two cubic centimeters of rabbit's blood add two cubic centimeters of ten per cent solution of cane sugar and then two cubic centimeters of the dog's serum.	
10.00 "	Now distinct darkening and laking.	
10.30 "	Still more laking. With microscope most of the corpuscles are seen to be decolorized. The ghosts are aggregated together very closely in clumps. The ghosts are round with smooth outlines as in water-laked blood. The unlaked corpuscles are often very irregular in outline, sometimes like amebæ with pseudopodia.....	
11.02 " (After twenty-eight hours)	40.75
	Top	42.32
		48.29
11.20 "	To two cubic centimeters rabbit's blood added two cubic centimeters of ten per cent sugar solution and two cubic centimeters of dog's serum which had been heated.	
1.27 P.M.	No laking..... (After twenty-eight hours)..... Top.....	41.49 42.39 49.13
9.46 A.M.	To two cubic centimeters rabbit's blood added one cubic centimeter of dog's serum.	
10.00 "	Distinctly darker.	
10.30 "	Dark and laked.	
10.55 " (After twenty-eight hours).....	48.10 52.81
1.33 P.M.	To two cubic centimeters rabbit's blood added one cubic centimeter dog's serum which had been heated.	
2.20 "	No laking	55.70
	(After twenty-six hours).....	56.21
11.25 A.M.	To two cubic centimeters of rabbit's blood added two cubic centimeters of heated dog's serum.	
1.41 P.M.	No laking	60.50
	Top (after twenty-eight hours)	80.99
12.15 "	Added to 0.5 gramme of cane sugar two cubic centimeters of dog's serum and two cubic centimeters of rabbit's blood. All sugar dissolved.	
1.15 "	Somewhat darkened.	
1.58 "	A little darkened, but not much..... (After twenty-eight hours many intact corpuscles, apparently little laking)	43.41 44.63
12.30 "	Added to 0.5 gramme of sugar two cubic centimeters of the heated dog's serum and two cubic centimeters of rabbit's blood. All sugar dissolved.	
1.50 "	No darkening or laking	44.79
	(After twenty-eight hours all corpuscles apparently intact)	46.01

EXPERIMENT XI. — The same rabbit's blood and dog's serum as were used in Experiment X., after standing forty-eight hours longer in the cold. Half saturated some of the dog's serum with cane sugar. Heated some of this sugar-serum to 62°–64° C. for fifteen minutes. For the unheated sugar-serum $\lambda = 40.43$, and for the heated, 40.89.

	λ .
To two cubic centimeters of the rabbit's blood added five cubic centimeters of the heated sugar serum. (Sixty-three minutes after mixture)	45.27
To two cubic centimeters of rabbit's blood added five cubic centimeters of the unheated sugar serum. (After fifteen hours still imperfectly laked)	44.71
To two cubic centimeters of rabbit's blood added five cubic centimeters of dog's serum without sugar. (Begins to lake almost at once.) (After fifteen hours in the cold laking complete)	70.81

EXPERIMENT XII. — Obtained rabbit's defibrinated blood by decapitation seven and one-half minutes before the beginning of the observations. For the defibrinated blood $\lambda = 43.04$; for the serum $\lambda = 82.30$. Percentage of serum, 68.4. For dog's serum obtained four days previously λ was then 82.04. Heated some of the dog's serum for ten minutes to 64° C., and then saturated it with sugar at room temperature. For the sugar-serum after heating $\lambda = 20.52$; for the sugar-serum before heating $\lambda = 22.10$.

Time.		A
11:32½ A.M.	To two cubic centimeters rabbit's blood added five cubic centimeters dog's serum.	
11:53 "	Not darkened nor laked.	
12:13 P.M.	Somewhat darkened.	
2:13 "	Laking only partial. (After forty-eight hours laking only partial) ...	71.61
	Top	81.77
2:15 "	To two cubic centimeters rabbit's blood added five cubic centimeters of the dog's serum after heating it to 64° C. for ten minutes. (After forty-eight hours no laking)	71.81
	Top	81.77
12:19 "	To two cubic centimeters rabbit's blood added five cubic centimeters of the serum saturated with sugar (not heated).	
1:30 "	Not darkened nor laked.	
2:15 "	Distinctly darkened. (After forty-eight hours laking is incomplete) ... (About same amount of Hb in the serum as in the blood to which unheated serum without sugar was added).	29.58
12:30 "	To two cubic centimeters rabbit's blood added five cubic centimeters of the sugar serum after heating. (After forty-eight hours, no laking)	29.07
	To dog's blood added six times its volume of the rabbit's serum; is not laked in nineteen hours. Dog's serum obtained seventeen days ago and kept in the cold produces no laking at all in sediment of rabbit's blood in nineteen hours.	

EXPERIMENT XIII.—Added to defibrinated dog's blood twice its volume of a solution containing forty-eight cubic centimeters of ethyl alcohol in one hundred cubic centimeters of one per cent NaCl solution. Laking is immediate. Added to a specimen of the same defibrinated blood twice its volume of a solution containing forty-eight cubic centimeters of alcohol in one hundred cubic centimeters of a twelve per cent solution of cane sugar. Laking follows in a few minutes, but not so soon as with the mixture of alcohol and salt solution.

Added to another portion of the same blood twice its volume of the alcohol and sugar mixture and then immediately powdered cane sugar. Laking takes place in between three and four minutes.

Added to fifteen cubic centimeters of the defibrinated blood ten cubic centimeters of a solution containing ten cubic centimeters of alcohol in one hundred cubic centimeters of one per cent NaCl solution. Let the mixture stand for twenty-two hours in the cold. No laking. It lakes at once on the addition of water or one per cent solution of NH_4Cl or of

more alcohol. The addition of a few drops of a three per cent solution of crude quillaia saponin (in one per cent NaCl solution) which has stood at room temperature for nine months also caused immediate and complete laking.

EXPERIMENT XIV. — To three cubic centimeters of dog's defibrinated blood added ten cubic centimeters of a ten per cent solution of NaCl. Heated to 64° C. The blood mixture darkens, but does not turn quite transparent. Rather suddenly (64° C. not having been exceeded) a considerable granular precipitate (serum-proteid whose coagulation temperature has been lowered by the presence of the salt) forms. The filtrate from this is transparent and contains much hemoglobin. Microscopically numerous intact corpuscles are seen, though the majority are evidently laked.

Washed a sediment of dog's defibrinated blood with one per cent NaCl solution to free it from serum. Centrifugalized, and suspended the sediment in saturated NaCl solution. Enough salt was added in substance to keep the solution saturated when it was heated. Heated for fifteen minutes to 62°–64° C. Rather suddenly some precipitate forms in it which is seen under the microscope to be granular. No doubt a precipitate of the remaining serum proteids. Now stopped heating. Under the microscope no intact corpuscles could be seen. By diluting with ten per cent NaCl solution and filtering it was shown that practically all the hemoglobin was in solution.

EXPERIMENT XV. — Dog's defibrinated blood was centrifugalized for many hours, the serum carefully pipetted off and measured, and the sediment used for laking experiments. For the sediment, λ is 3.88; for the serum, 86.22; for the defibrinated blood, 29.48. The percentage of serum in the sediment, deduced from the formula $p = \frac{\lambda(b)}{\lambda(s)} (174 - \lambda(b))$,¹ is 7.7, and the percentage of corpuscles 92.3. The general accuracy of this result was controlled by the measurement of the amount of serum actually recovered. In the defibrinated blood the calculated percentage of corpuscles is 50.7. The original blood was therefore richer in corpuscles than the average. Complete laking is caused in this sediment by crude saponin in substance, sodium taurocholate in substance, ethyl alcohol, ether, amyl alcohol, chloroform, and heating for ten minutes to 62°–64° C. The addition of five-tenths cubic centimeter of distilled water to two cubic centimeters of the sediment causes very incomplete laking, the great majority of the corpuscles retaining their hemoglobin.

Laking is also caused by the reagents mentioned when the sediment

¹ Jour. of Physiol., Vol. xxiv, p. 356.

has been allowed to dry partially on slides, in which case, of course, the percentage of water between the corpuscles must be still less than seven to eight.

EXPERIMENT XVI. — Added to thirty-five cubic centimeters of pregnant rabbit's defibrinated blood, obtained by decapitation two hours before, twenty-five cubic centimeters of a four per cent solution of formaldehyde in one per cent NaCl. After sixteen hours removed thirty cubic centimeters of the clear liquid. Added one hundred and forty cubic centimeters of one per cent NaCl, mixed and centrifugalized. The sediment of the formaldehyde blood is not laked by the addition of much water, as shown by the microscope. Decanted off the clear liquid from the formaldehyde sediment after centrifugalization. Added more NaCl solution and centrifugalized again. Both times the sediment was quite compact, and all the liquid was allowed to drain off by inverting the tubes. Added now to the sediment as much one per cent NaCl as is necessary to make it sufficiently thin for conductivity measurements. Call this mixture A.

	A
A	42.32
To five cubic centimeters of A added ten cubic centimeters of dog's serum (from blood drawn two days ago).	
(After eighteen hours)	71.81
Top	86.22
(After seventy-two hours) Bottom	59.79
To five cubic centimeters of A added ten cubic centimeters of dog's serum after heating it to 64° for ten minutes.	
(After eighteen hours)	68.70
Top	86.52
(After seventy-two hours) Bottom	57.88
The dog's serum	79.97
The heated dog's serum	80.73

EXPERIMENT XVII. — Added to dog's defibrinated blood two-thirds of its volume of a four per cent solution of formaldehyde in eight-tenths per cent NaCl solution. Let it stand for three days. Then separated the sediment of corpuscles, and suspended it in its own volume of a one per cent NaCl solution. For this suspension $\lambda = 78.73$. Heated to 63° C. and again measured the conductivity. Again $\lambda = 78.73$.

EXPERIMENT XVIII. — To dog's defibrinated blood added three-fourths of its volume of a four per cent solution of formaldehyde in NaCl solution. Let it stand a week in the cold. Then separated the sediment, washed with NaCl solution, centrifugalized again, and suspended the sediment in NaCl solution. For the suspension $\lambda = 60.94$. For some of the suspension after heating to 62° – 64° C. for eight minutes $\lambda = 59.93$. The addition of four-tenths cubic centimeter of saponin solution in NaCl solution (λ being 133.42 for the saponin solution) to five cubic centimeters of the suspension raised its λ to 79.47, an increase far too great to be due to the electrolytes contained in the saponin solution.

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PRELIMINARY NOTE ON SOME EFFECTS OF TOBACCO ON THE
TISSUES OF RABBITS.

I. ADLER, M.D.

It is not the object of the studies of which this is a preliminary and very fragmentary report to offer a contribution to the toxicology of tobacco or nicotine. They form part of a larger series undertaken for the purpose of investigating the etiology and very early stages of those interstitial and vascular lesions commonly grouped together under the name of arterio-sclerosis. For reasons which need not be stated here, it was found necessary to investigate the effects of chronic tobacco poisoning. The experiments are still in progress and not near a definite conclusion; nevertheless the results thus far obtained may be of some interest. All the experiments are made upon rabbits. The animals are kept in box-stalls so that they can move about freely though confined within proper limits. The stalls are littered with excelsior which the rabbits will not eat, and they are thus compelled to subsist entirely upon the food given them. No stomach tubes or other means of artificial feeding are resorted to; this, in order to avoid any injury and approximate as nearly as possible to the natural conditions. The animals are fed exclusively upon fresh cabbage. Each rabbit receives once a day a sufficient quantity of cabbage chopped fine and carefully mixed with an infusion of tobacco. No attempt at exact dosage is made. The infusion is prepared by breaking up cheap cigars (two for five cents) and allowing them to soak over night in a pint of water which is then filtered and carefully mixed in increasing quantities (from three ounces to half a pint) with the day's allowance of cabbage. At the

beginning two cigars to the pint are used, later three, and still later four cigars, so that in the latter months of the experiment each animal consumes a very large quantity of tobacco juice. In no case has the food been refused, and all animals without exception have eaten every morsel that has been given them. At first they appear somewhat nauseated and sick, though they move about freely and continue to eat. After a very few days they are perfectly normal, lively and well. They do not appear to lose in weight, feed greedily, and all their functions are absolutely normal. A female rabbit is at present under observation that has been fed upon tobacco infusion in the manner described for six months and who consumes at present daily a half pint of infusion made from four cigars. During this time she has become pregnant and given birth to seven young, everything passing off quite normally. She has nursed her offspring, and is at present fat and apparently entirely healthy. All the rabbits are full grown, but not old. Their exact age could not be ascertained. Thus far no animal has died, but all appear quite healthy until killed for examination.

A rabbit killed after three weeks of the treatment just described shows no signs of disease anywhere, even upon closest inspection. All the organs appear entirely normal. The stomach is full as usual, its mucous membrane and the other coats appear healthy. The liver is of normal size, color, and consistency. The kidneys are normal. The bladder is filled with clear, normal urine. Pieces of the liver, kidneys, spleen, pancreas, stomach, intestines, heart, and aorta are, while still warm, placed in fixing solution, afterwards hardened and embedded in paraffin and celloidin. The best results are obtained with Zenker's solution for paraffin and ten per cent formalin for celloidin. Careful microscopic examination with the aid of various stains shows all the organs mentioned to be perfectly normal, with the exception of the liver, the organ which in experiments of this type is usually the first to show signs of disease. The arrangement of the various component parts of the liver is quite normal, as also are the liver cells. There is no con-

gestion and no degeneration, but surrounding the smaller branches of the interlobular vessels and especially the smaller bile-ducts there is a marked though not very extensive conglomeration of round cells of the type known as granulation cells. This is very distinct, the more so as everything else is absolutely normal. The central vein of each lobule is entirely free.

An animal killed after two and a half months, apparently in perfect health, presents a somewhat different picture. While all the other organs, macroscopically and microscopically, show no signs of any lesion whatsoever, the liver at once attracts attention. It is decidedly larger than normal, is somewhat paler in color, distinctly granular in appearance, of firmer consistency, and causes a somewhat gritty sensation when cut with a knife. The normal liver of the rabbit, as is well known, contains but very little interlobular fibrous tissue, and lobulation is therefore but faintly indicated. In this liver lobulation is very pronounced. There is a very distinct increase of interlobular tissue throughout the entire organ, visible in thin sections with the naked eye. The proliferation of the fibrous tissue follows the track of the portal vessels and the bile ducts. In many places the fibrous tissue is rather firm and hard, contains comparatively few cells and much elastic fiber. In others it is loose and delicate, carrying abundant round and fibroplastic cells. There is also evident proliferation of the smaller bile-ducts somewhat analogous to cirrhosis of the human liver. The proliferation is well marked within the lumen; the ducts themselves being frequently closely surrounded by fibrous tissue. The new formation of connective tissue does not extend into the interior of the lobules; it is strictly confined to the interlobular spaces. A most careful study fails to detect the slightest evidence of any lesion of the parenchyma. The liver cells are absolutely normal and even in the close neighborhood of the interlobular spaces and the newly forming fibrous tissue show no signs of degeneration or proliferation. The process is restricted entirely to the interstitial tissue and the interlobular spaces.

A rabbit killed while in apparently healthy condition after four months of tobacco treatment presents to the naked eye about the same appearance as the preceding animal. The liver is still large, of the same grayish-red color, but distinctly more granular, harder, and very gritty under the knife. All other organs appear quite normal. The microscope shows advance of the interlobular process. The fibrous tissue has increased in bulk and extent. It begins, very sparingly it is true, but distinctly, to enter between the cells of the lobules. There is a very abundant infiltration of the fibrous tissue with round cells. The new formation and proliferation of bile-ducts has assumed larger proportions. Here and there a small artery can be seen with proliferation of a portion of its intima, a typical picture of endarteritis nodosa. But notwithstanding all this progressive interstitial activity, the parenchyma, the liver-cells, and the central vein remain entirely untouched. There is no change in the cells, no degeneration of any kind; they are thus far absolutely normal and in no wise participate in the morbid process. Stomach, gut, spleen, and pancreas are normal. In the kidney, however, there may possibly be some incipient change. I have the impression that the first beginnings of an interstitial proliferation of tissue can be made out in several places in the cortex. The changes, if any, are, however, so slight and the subject is so difficult and complicated that it seems better to leave this matter in suspense awaiting the further development of the experiments. The same holds good for the heart. While the hearts of the preceding animals are unquestionably entirely normal, there is an impression that in the heart of the last animal there may be beginning round-cell infiltration and proliferation surrounding the smaller blood-vessels and capillaries. This seems to be particularly noticeable in the outer layer of the heart muscle, though the epicardium and the muscular tissue immediately adjoining it appear quite normal. Upon this subject also I cannot speak with any assurance; it is as yet merely an impression and may be quite erroneous.

This is as far as the experiments have progressed up to

the present. Whether this interstitial process in the liver is progressive and will ultimately affect the parenchyma; whether any analogy with certain forms of cirrhosis of the human liver will be established; whether other organs will successively participate in this process of interstitial fibrosis, and if so, to what extent; whether the vascular system will also be affected; these and many other questions await solution from further observation, the results of which I hope to report at some future time.

I have ventured to present this preliminary note at this early stage of experimentation because it appears that at least one fact even now may be considered as reasonably certain.

A very considerable amount of work has been done on so-called experimental liver cirrhosis and the effects of chronic poisoning on the various organs. The lesions due to alcohol, phosphorus, lead, bacterial and other toxins have again and again been investigated. There is, however, still disagreement as to results. Where considerable interstitial hyperplasia occurs, as in phosphorus and alcohol poisoning, the liver-cells are also affected to such an extent and at so early a stage of the process that it is often impossible to decide which is primary, the interstitial or the parenchymatous affection. It thus happens that opinions differ greatly, some looking upon the interstitial proliferation as caused by and secondary to the primary destruction of the parenchyma cells, while others consider the interstitial fibrosis as primary, and the cell degeneration of the parenchyma as due to the alterations of the interlobular tissue and consequent pressure upon the lobules, interference with vascularization, nutrition, etc. (See Siegenbeek van Heukelom, Ziegler's Beiträge, Vol. XX., where a very complete and critical discussion of the whole subject up to 1896 may be found.) An unequivocal answer to the question— are there any toxic agents which primarily, and for a time at least, solely, affect the interstitial and fibrous tissue? — is therefore of some importance. And this not only with reference to cirrhosis of the liver, but also with regard to more general conditions, arterio-sclerosis, for

instance. In many of the experiments under consideration there is also some uncertainty as to just how much of the observed lesions is due to the toxic agent employed and how much to gastritis, enteritis, sepsis, and other complications incidentally produced. Comparatively very little experimental work has been done with tobacco. In the literature accessible to me I find only Kohos (*Bulletin Med. Paris*, 1897, XI., p. 348) employing methods similar to ours. He gave dogs, guinea-pigs, and rabbits three to four times daily twenty to twenty-five centigrams of tobacco in substance mixed with their food, and continued this for about two months. His description of the histological changes in his animals, in the very brief notice available, is too short and incomplete to permit any assured deductions. He finds grave changes both in the liver cells and in the fibrous tissue. In our own experiment we may first of all remark in passing, the extreme chronicity of the process. This accords well with what is thus far known of chronic tobacco poisoning. Every one who has given any attention to the subject has remarked upon the tolerance that is shown, both by the human being and by animals, to the long-continued abuse of tobacco, in contradistinction to the extremely rapid and fulminating course of acute nicotine poisoning. It has not been our object to attempt to determine which of the many constituents of tobacco juice acts as the toxic agent. This would be most interesting work for the chemist. It is quite possible that it is nicotine. Kohos, in his experiments, demonstrated the presence of nicotine in the liver, spleen, heart, and intestines even after tobacco feeding had been interrupted for ten days. It may be that some of the organic acids produce the toxic effects. In this connection it is well to remember that Boix (*Le Foie des Dyspeptiques*, Thèse de Paris, 1894) succeeded in producing a cirrhotic condition of the liver in rabbits by feeding them with butyric, acetic, and valerianic acids during periods extending from sixteen to three hundred and eighty-seven days. It is quite possible, too, that inorganic substances may play a toxic role, and perhaps a combination of a number of substances may be

necessary to produce just this effect. But however this may be, this much may be safely inferred, that we have in tobacco infusion, administered as described, a toxic substance, probably one of many, which, acting with extreme chronicity, exerts its influence, at least in the earlier stages, exclusively upon the interstitial and fibrous tissue, uncomplicated by any parenchymatous degeneration or any gastric or intestinal lesion.

ON THROMBI COMPOSED OF AGGLUTINATED RED BLOOD
CORPUSCLES. — PRELIMINARY COMMUNICATION.¹

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The study of the phenomena of agglutination has extended far beyond bacteria. Beginning with Bordet's observations on the production of hemolysins, the agglutination of red blood corpuscles by such hemolytic sera has been carefully noted. Long prior to these observations the same appearances had been seen and described by Weir Mitchell as one of the characteristic phenomena of the action of snake venoms upon the corpuscular elements of the blood. That the appearance had also been observed in the course of the study of the globulicidal action of normal sera upon alien red corpuscles is highly probable although no especial interest seems to have been manifested in the phenomenon and no especial attention given to its occurrence.

The more recent studies upon agglutination and lysis of bacteria have shown that these effects are produced by two different groups of substances, and very recently Flexner and Noguchi have been able to prove that the agglutinating principle for blood corpuscles in snake venoms is distinct from the dissolving substance. That the same is true in ordinary hemagglutination, as observed in normal sera, is easily demonstrable by selecting a serum that possesses strong agglutinating power for red corpuscles while being almost or wholly devoid of dissolving action. The serum of the horse, for example, agglutinates strongly human and rabbit's corpuscles, and only very rarely causes minimal and imperfect solution of them.

That a serum may be agglutinating for red corpuscles of a similar animal species has also been shown. Such isoagglutinins are, however, relatively uncommon and of weak activity.

¹ Presented March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists at Cleveland, Ohio;

The great interest manifested in the study of hemolysis within the past twelve months has brought to light the interesting fact that products of some bacterial cultures are hemolytic. Whether or not these cultures are also hemagglutinative has been little studied. That the activity of the cultures depends upon soluble products and not upon the bacteria themselves is readily demonstrable through the use of sterile filtrates.

Temperature plays an important part in the development of the phenomena of hemolysis. At temperatures approaching freezing it is usually absent. Agglutination, on the other hand, is not prevented by such low temperatures. My observations upon the effects of hemolytic culture filtrates, such as those derived from growths of *B. typhosus* and *B. pyocyaneus*, carried out at zero temperatures, show that agglutinines may also be present, but that they are of low activity; and certain cultures of *S. pyogenes aureus* may be agglutinative and entirely non-hemolytic.

Kobert and his pupils, as well as Ehrlich, have described agglutination of red corpuscles through the action of several phytalbumoses, the best known and most extensively studied ones being ricin and abrin. The freedom from fibrin of the coagulum produced in blood by these bodies was first pointed out by Kobert; and Ehrlich attributed a part, at least, of their pathological effects to such coagulative thrombi.

The natural occurrence of thrombi composed of agglutinated red corpuscles in human and animal pathology seems not to have been noted. That the conditions of their experimental production often occur in natural disease is self-evident so far, at least, as bacterial infection goes. Whether still other conditions of their origin occur naturally we are not so definitely informed. The phenomenon of isoagglutination indicates, however, that they may occur; and I think that there are already at hand observations upon thrombi free from coagulation, that makes such occurrence all but certain.

AGGLUTINATIVE RED-CORPUSCLE THROMBI IN BACTERIAL DISEASE.

My attention was first drawn to the occurrence of a special kind of thrombosis, caused by agglutinated corpuscles, from the detection of such a thrombus, in the dilated veins in the intestinal submucosa, in the immediate neighborhood of an ulcerated typhoid patch in the ileum. The vessels occluded were, in the sections that I examined, of medium size and two in number. The appearance of the thrombi was distinctly peculiar and, at first sight, puzzling. Leucocytes took no part in the process, and, in keeping with the disease, the polymorphonuclear varieties were few in number. Such leucocytes as were present were of the small mononuclear variety. Fibrin also was present in islands in very small amount, attached usually to the walls of the vessels. On the other hand, the vessel was quite occluded by a conglutinated mass made up of globules of different sizes showing different degrees of refraction and varying staining properties. Careful study readily supplied the conviction that the mass was composed of red corpuscles, altered in form, adhesiveness, and staining properties. Every transition was found from normal red corpuscles to the main agglutinated mass in which the corpuscular outline was lost and a higher degree of refraction existed.

The conviction that the thrombus consisted of agglutinated red corpuscles led to a further search being made for similar occurrences. About twenty additional cases of typhoid fever were studied. For this purpose sections from all available organs were made. In no other instance was anything so convincing found; but appearances were seen in the intestines and lungs in several other cases that left little doubt that they also depended upon agglutinated red corpuscles.

Unmistakable evidences of similar formations were found in sections of a lung in which bronchiectasis and broncho-pneumonia existed. The bacterial development in the tissue was massive.

Sections of the stomach from a case of carbolic acid poi-

soning also showed capillary and larger vascular thrombi composed of such agglutinated corpuscles.

The nature of the hyaline glomerular thrombi occurring in the kidney in infectious disease — both natural and experimental — has not been satisfactorily settled. That some of them are fibrinous seems established. Others, however, fail to give the staining or other reactions of fibrin, are non-fibrillated, nearly or quite homogeneous, and of a pale yellow color. In human beings these thrombi have been seen in pneumonia, diphtheria, and other acute infectious diseases; and in animals they are frequently found in experimental infections, such as hog cholera and diphtheria, and in pneumococcus and staphylococcus aureus infections.¹ A re-examination of an old series of kidney sections from a kitten inoculated with *B. diphtheriae* gave support to the view that the hyaline glomerular thrombi consist of agglutinated corpuscles.

AGGLUTINATIVE RED-CORPUSCLE THROMBI IN OTHER THAN BACTERIAL DISEASE.

For the study of the possible occurrence of similar thrombi to those just described in a disease probably not of bacterial origin there was available a case of eclampsia in which the hepatic lesions (consisting of necrosis and hemorrhage) were abundant. Several pieces of this liver were sectioned serially by Mr. Havens, who hopes to report later upon the findings in detail. In this place it may be mentioned that evidence of the occurrence of capillary thrombi composed of red corpuscles was obtained. The thrombi are found in the neighborhood of the areas of necrosis and hemorrhage.

A study of the description given by Klebs of the thrombi in glaucoma strongly suggests the idea that they may be of this nature. The same is true of the hyaline thrombi described by Recklinghausen in pulmonary infarction. Indeed, it is not improbable that many of the so-called hyaline

¹ That these thrombi are at times composed of red corpuscles had previously been suggested by Welch and by Klebs.

thrombi may be of red corpuscular origin, having arisen through agglutination.

EXPERIMENTAL NON-BACTERIAL AGGLUTINATIVE THROMBI.

A series of experiments was undertaken (1) to compare experimentally produced agglutinative thrombi with those occurring in natural disease, and (2) to determine the nature of the thrombi produced in the heart and great blood-vessels by chemical agents and alien globulicidal blood sera.

For the first purpose, ricin was chosen. Two series of experiments were carried out. In (*a*) the red corpuscles of the rabbit were agglutinated *in vitro* with ricin, washed and injected into the ear vein of the rabbit. In (*b*) ricin in solution was injected into the ear vein of the same species of animal. The injection of corpuscles agglutinated *in vitro* causes rapid death of the rabbit, and thrombi are easily found in the vessels of the lungs. When death is delayed for five or ten minutes polymorphonuclear leucocytes may have made their way in small numbers into the agglutinated masses. The intra-venous injection of minimal fatal doses of ricin is followed by the death of the animals in from eighteen to twenty-four hours. The lesions are characteristic; and in the swollen and hemorrhagic intestine and lymphatic glands agglutinative thrombi may be found. They are essentially alike in both series.

For the study of chemical thrombi ether was injected into the ear vein of the rabbit. Death occurred in three or four minutes. The animal is opened immediately, when the heart is found pulsating feebly. On opening the heart the right side is found to be filled with a dark, soft, conglutinated clot, extending into the pulmonary vessels. The fluid portion of the blood is laked in appearance. Sections of the clot show a part of the corpuscles in the state of shadows — or ghosts — and others to be agglutinated. Fibrin is absent.

Dog's blood serum is strongly globulicidal for rabbit's blood, and if injected intravenously in quantities approaching

one per cent of the body weight of the animal, it frequently causes death in a few minutes. Death is preceded by convulsions. On opening the animal at once appearances similar to those described in the case of ether poisoning are observed. Sections prepared from the clot in the heart and pulmonary vessels show absence of fibrin and the occurrence of agglutinated red corpuscles.

CONCLUSIONS.

(1.) The agglutination of red corpuscles *intra vitam* is not uncommon in infectious disease in man and animals.

(2.) A special variety of thrombi is produced through this agglutination which may be denominated agglutinative thrombi.

(3.) When such thrombi are old, or when the agglutination is compact, they may present appearances to which the name of "hyaline thrombi" has been applied.

(4.) Other alterations of the blood than those arising in infectious disease may bring about agglutinative thrombosis, the nature of this alteration being little understood.

(5.) Poisons which destroy corpuscles rapidly are provocative of agglutinative thrombosis.

(6.) The so-called "fibrin ferment thrombi" are probably nothing else than agglutinative thrombi.

EXPERIMENTS ON THE PERMEABILITY OF THE BERKEFELD
FILTER AND THE PASTEUR CHAMBERLAND BOUGIE TO
BACTERIA OF SMALL SIZE.

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There seems to be a general belief among bacteriologists that if the virus of a disease passes through the pores of a Berkefeld filter, it must be ultramicroscopic. This idea no doubt arose from the fact that the cause of foot and mouth disease, as shown by Löffler, and more recently, the virus of yellow fever, as demonstrated by Reed and Carroll, will pass through the pores of the Berkefeld filter.

In September, 1899, in connection with some work on guinea-pig pneumonia,* Dr. Theobald Smith noted that the small, actively motile bacillus¹ which causes this disease would pass through the pores of the small Berkefeld filter (No. 5). This filter is the one most generally used in filtering small amounts of serum or toxin.

Briefly the findings were as follows:

A one hundred cubic centimeter flask was inoculated from an agar culture of the bacillus from pneumonia in guinea-pigs. Four days later seventy cubic centimeters were filtered through a small Berkefeld filter and collected in ten cubic centimeter lots in seven sterile test tubes. The organism grew in all the tubes excepting the first two.

In connection with the question of the permeability of filters an interesting fact was demonstrated, in 1892, by Dr. Theobald Smith and Dr. V. A. Moore.² In these experiments a twenty-four-hour bouillon culture of the bacillus of hog cholera was placed inside a sterile Pasteur Chamberland bougie and the fluid forced through into the surrounding test tube. In one instance a growth of hog cholera bacilli appeared in the surrounding fluid in ten days, in the other, in five days. Thus showing that the organism is able to grow through the filter wall.

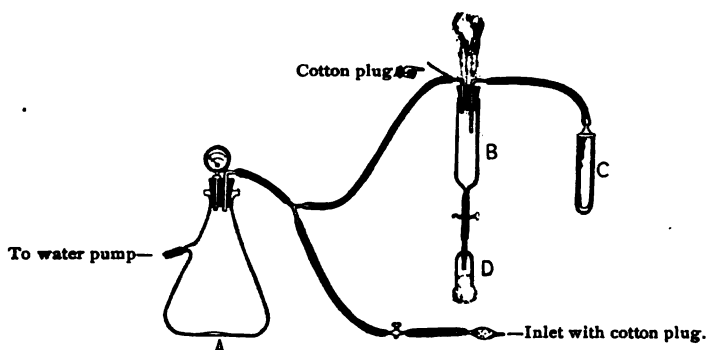
At the suggestion, and under the direction of Dr. Smith,

* A study of this organism and the disease produced in guinea-pigs was begun early in 1899, but has remained unfinished. Dr. Smith hopes to publish notes upon it during the year.

the following piece of work was undertaken to re-examine these findings:

APPARATUS AND METHOD OF FRACTIONAL FILTRATION,

—The filtration was performed by means of negative pressure and the apparatus was arranged as shown in the accompanying cut.



A water-pump was connected with an ordinary aspirator flask (*a*), which was closed with a perforated rubber stopper, and provided with a vacuummeter and a glass outlet tube. The latter was connected by means of pressure tubing to a Y-shaped brass tube which connected, on the one hand, with an inlet tube which was furnished with a stop-cock and terminated in a glass bulb packed with sterile absorbent cotton; and on the other, with the receiving tube (*b*). The receiving tube was closed with a perforated rubber stopper through which two bent glass tubes passed. One of these was connected with the pressure tubing from the vacuum flask and its outer arm was packed with absorbent cotton. From the other tube a connection passed to the Pasteur or larger Berkefeld filter (*c*). The receiving tube was connected below, by rubber tubing, with a protected mouth-piece according to Maassen (*d'*). This connection was provided with a screw-cock. The rubber connections throughout were of heavy pressure tubing excepting that between the receiving tube and the protected mouth-piece, which was of ordinary gum rubber.

When filtering with the Pasteur, or larger Berkefeld, the arrangement shown in the cut for the Pasteur was used. When the small Berkefeld was used it was mounted, within its glass mantle, upon the receiving tube as shown by the dotted lines. Just before use the receiving tube with its connections was sterilized in the autoclave at 111° C. for twenty minutes. The larger and small Berkefeld filters in their glass mantles, plugged with cotton, were sterilized in the autoclave as above. The Pasteur Chamberland bougie, inside a large test tube and protected with cotton, was sterilized in the hot-air sterilizer for two hours at 130° C. The filters were all new and apparently in perfect condition.

The fluid culture was transferred to the large test tube enclosing the larger Berkefeld or Pasteur bougie and to the mantle enclosing the small Berkefeld with a sterile pipette provided with a rubber bulb. Then the water was turned on, and as the fluid passed through the filter enough of the culture was added from time to time to keep the bougie covered as much as possible. The vacuum obtained as registered by the vacuummeter, unless otherwise mentioned, was 600 mm. The fluid filtered at a much lower pressure, but as a rule little came over into the receiving tube before a pressure of 600 mm. was reached. When the required amount of the filtrate had passed over into the receiving tube, the pressure was relieved by turning the stop-cock on the inlet tube and allowing the air to enter through the glass bulb packed with absorbent cotton. The water was then turned off and the filtrate collected in sterile test tubes.

EXPERIMENTS IN FILTRATION. — *A.* With organism from pneumonia in guinea-pigs.

An actively motile bacillus, 0.5μ wide and about 0.7μ long.

1. Berkefeld filter, small size, No. 5.

a. Three-day bouillon culture. About 125 cc. filtered ; 25 cc. filtered at a time, collected separately in each of five sterile test tubes and placed in the incubator at 37° C. Two days later tubes 4 and 5 showed cloudiness with the forma-

tion of a characteristic iridescent pellicle on the surface. Microscopically a small, actively motile bacillus was seen. The tubes were watched eight days, but no growth appeared in the first three tubes. Reaction of the first filtrate 0.8 acid to phenolphthalein.

b. Twenty-four-hour-bouillon culture. About 100 cc. filtered; 20 cc. filtered at a time, collected separately in each of five sterile test tubes and placed in the incubator at 37° C. Two days later growth appeared in the fifth tube -- pellicle formation, and a small, actively motile bacillus in the hanging drop. The first four tubes remained clear for five days. Reaction of first filtrate 1.1 acid to phenolphthalein.

II. Berkefeld filter, larger size (No. 8, marked 3), not washed before sterilization.

Filtered about 200 cc. of a mixture of a twenty-four and forty-eight hour growth in bouillon; 20 cc. filtered at a time, collected separately in each of ten sterile test tubes and placed in the incubator at 37° C. Pressure 550-600 mm., fluid began running over at 400 mm. In three days no growth appeared. The reaction of tubes 1, 5, and 10 was about 0.8 acid to phenolphthalein. I inoculated tubes 3, 6, and 9 to see if the fluid exerted a germicidal action and obtained good growth in twenty-four hours.

III. Pasteur-Chamberland bougie, grade F.

Twenty-four and forty-eight hour bouillon cultures were filtered on three different occasions, a new bougie being used each time. Seven to twenty cubic centimeters collected separately in each of five to ten test tubes and placed in the incubator at 37° C. The filtrates watched for four to ten days remained clear of growth, excepting the first tube of one set which contained a biscuit-shaped diplococcus (outside contamination). The reaction of the filtrates was between 0.9-1.3 acid to phenolphthalein.

B. With *Bacillus coli communis*.

I. Berkefeld filter, small size, No. 5. Used in preceding experiment; boiled in five per cent solution of sodium carbonate and washed until no longer alkaline to phenolphthalein.

a. Twenty-four-hour bouillon culture. About 125 cc. filtered; 15–20 cc. filtered at a time, collected separately in each of six sterile test tubes and placed in the incubator at 37° C. Filtered fast for the first two tubes, then dropped slowly. Tubes remained clear after six days.

b. Twenty-four-hour bouillon culture. About 125 cc. filtered at pressure of 400–650 mm.; 20 cc. filtered at a time, collected separately in each of six sterile test tubes and placed in the incubator at 37° C. No growth; tubes discarded after four days.

II. Berkefeld filter, larger size (No. 8, marked 3). New; washed before sterilization.

a. Twenty-four-hour bouillon culture, diluted one-half with sterile normal salt solution and placed in incubator for half an hour. On microscopical examination the bacilli appeared actively motile. About 125 cc. filtered at pressure of 400 mm.; 30 cc. filtered at a time, collected separately in each of six sterile test tubes and placed in incubator at 37° C. All tubes remained clear after six days.

b. Twenty-four-hour bouillon culture. About 100 cc. filtered at pressure of 400 mm.; 15 cc. filtered at a time, collected separately in each of six sterile test tubes and placed in incubator at 37° C. Came through very foamy, as the fluid did not completely cover the filter bougie. Tubes still clear after four days.*

EXPERIMENTS SHOWING THE GROWTH OF BACTERIA THROUGH THE PORES OF FILTERS. — This was done in order to determine how soon these two organisms would grow through the wall of the filter. The filter bougie was suspended in a test tube of such size as to completely cover the filter and leave a clear space or reservoir of an inch or two at the bottom. Then, by suction of the water pump,

* The objection might be raised that the filtrates in some cases might have been unsuited for further bacterial growth. But this is unlikely. The initial reaction of the bouillon used was 1 to 1.5 per cent acid to phenolphthalein. None of the cultures were old enough to have exhausted the nutrient materials present as shown by the final titrations of some of the filtrates which varied between 0.8 to 1.3 acid to phenolphthalein. Then, in experiment A II., subsequent inoculation of the filtrate proved it capable of supporting a vigorous growth.

bouillon was drawn into the filter. On standing a few minutes the fluid surrounding the bougie could be seen to change its level — evidently showing that the fluid on the outside and inside sought the same level. When, finally, the fluid on the outside covered the lower half of the filter, the openings at the upper end were enveloped in cotton and the whole sterilized in the autoclave at 111° C. for twenty minutes. On cooling, one to two cubic centimeters of a bouillon culture of the desired organism was introduced, with a sterile pipette, into the interior of the filter and the whole placed in the incubator at 37° C. Clouding of the clear fluid below the filter bougie was taken to indicate growth.

Results: The bacillus of guinea-pig pneumonia grew through:

1. The small Berkefeld, No. 5 in 3 days.
2. The larger Berkefeld, No. 8 in 1 day.
3. The Pasteur-Chamberland (F) in 9 days.

Microscopical examination of the clouded fluid in these cases showed the presence of a small actively motile bacillus.

Bacillus coli communis grew through:

1. The larger Berkefeld, No. 8 in 1 day.
2. The Pasteur-Chamberland (F) in 6 days.

Microscopical examination showed a feebly motile bacillus which produced gas bubbles in a gelatin shake culture, coagulated milk in forty-eight hours, and produced gas in the saccharose fermentation tube.

SUMMARY. — 1. The bacillus-producing pneumonia in guinea-pigs will pass through the pores of the small Berkefeld filter, No. 5, under the conditions of the experiments noted. It did not pass through the larger Berkefeld, No. 8, perhaps on account of the greater thickness of the walls, the relatively small amount of the fluid passed through, and the somewhat diminished pressure. Nor did it pass through the Pasteur-Chamberland bougie (F). It is probable that these filters, especially No. 5, will be found even less efficient

when very turbid, concentrated cultures are passed through. This was the case in Smith's experiment cited above.

2. *Bacillus coli communis* did not pass through any of the above filters.

3. *Bacillus coli communis* and the organism from pneumonia in guinea-pigs grow through the walls of the Berkefeld filter very rapidly; through the walls of the Pasteur (F) rather slowly.

4. It seems justifiable to conclude that if the virus of a disease passes through the pores of the Berkefeld it is not necessarily ultramicroscopic. Further work with organisms of various sizes might help in defining the size of the pores of such a filter and determine whether various filters of the same make have the same sized pores or not.

5. It would seem advisable that workers should make a preliminary test of filters, by employing some such minute organism, in order to determine more accurately the efficiency of the filter used.

REFERENCES CITED IN THE TEXT.

1. For literature upon this organism the reader is referred to the following articles :

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 - b. Strada, F., und Traina, R. Ueber eine neue Form von infektiösen Lungenkrankheit der Meerschweinchen. Centralblatt für Bak. Par. und Infek., Bd. xxviii, pp. 635-648.
 - c. Martini. Ein gelegentlicher, durch Inhalation übertragbarer Erreger der Lungenentzündung bei Meerschweinchen, *Bacillus pulmonum glutinosus*. Archiv. für Hygiene, 1900, Bd. xxxviii, Heft. 2.
2. Zur Prüfung der Pasteur-Chamberland-Filter, von Dr. Theobald Smith und Dr. V. A. Moore (September, 1892). Cent. für Bakt. und Parasit., Bd. xii, pp. 628-629.

RESEARCHES ON THE ACTION OF TUBERCULIN ON RABBIT'S BLOOD.

HUGH M. KINGHORN, M.D.

*(From the Saranac Laboratory for the Study of Tuberculosis. Dr. E. L. Trudeau, Director.)**Introduction.*

This research formed a part of the work done by Doctors Baldwin and Levene on the action of proteolytic enzymes on bacterial toxines.¹ One of their objects was to investigate whether proteolytic enzymes neutralized or digested toxines. They limited their work to diphtheria and tetanus toxines and to tuberculin. As a result of their experiments, they found that diphtheria and tetanus toxines are both digested and not simply neutralized by the three proteolytic enzymes, and thereby rendered inert without apparent selectivity. They found that trypsin and pepsin digestion weakened the specific activity of tuberculin; that trypsin on prolonged digestion is able to destroy all the activity of tuberculin, while the same could not be achieved by pepsin. They found also that in some cases the trypsin digested tuberculin, where the digestion was not too prolonged, failed to produce a local reaction on the rabbits with a localized tuberculosis of the eye, and failed to kill tuberculous guinea-pigs, but still possessed a fever-producing power. On the other hand, they found that pepsin-digested tuberculin still possessed the power of producing local reaction on the eye rabbits, and retained its toxic power for tuberculous pigs, though weakened. From the above facts they concluded that tuberculin is a specific substance of the nature of a nucleoproteid, which would account for its being more easily destroyed by trypsin than by pepsin, since nucleo-compounds are more resistant to peptic digestion.

The conclusions of Baldwin and Levene were based, as already mentioned, on the observations of the temperature

and local reaction produced by the injections. There is, however, one other constant symptom during a tuberculin reaction, namely, a change in the character of the blood-leucocytosis. This change is also produced by the injection of nucleo-compounds. It seemed of interest, therefore, to examine the blood of tuberculous animals when injected with tuberculin digested with the different proteolytic enzymes. If only of a proteid nature, it would seem reasonable to expect that on digestion it would fail to cause the blood changes which are produced by ordinary undigested tuberculin. On the other hand, if its action on the blood is easier abated by digestion with trypsin than with pepsin, the assumption would seem justifiable that tuberculin is a nucleo-compound.

Into the anterior chamber of the right eye a drop or two of a thin emulsion of tubercle bacilli of low virulence was injected on October 26, 1900. Those rabbits were chosen in whose eyes there were well-defined tubercles without much congestion. After the third week following the inoculation the eyes were usually in a condition suitable for the experiment.

Some injections were given at eight A.M., others at noon. Eight A.M. was more suitable, as both local and general reactions could be more closely observed during the daylight. The injections were given subcutaneously.

The blood was obtained by puncturing the marginal vein of the rabbit's ear. The leucocytes were counted by the Thoma-Zeiss hemocytometer. Coverslip preparations were heated in the hot air bath to 140–150° C., and then stained by Ehrlich's triacid stain. The blood was taken from the rabbits at the same time each day — twelve noon — while the rabbits were fasting.

THE CHANGES THAT FOLLOWED INJECTIONS OF KOCH'S OLD TUBERCULIN. — With this tuberculin there were seven experiments. Marked reactions, both constitutional and local, occurred in all the rabbits injected.

Local Reaction.

When a marked reaction resulted, congestion of the eye was observed in four hours or earlier. If the reaction were not so marked, congestion of the eye was not observed till the following day. When thus delayed, the congestion was slight and passed off quickly.

The height of the reaction was reached in from eight to twelve hours after the injection and lasted during the following day. About thirty-six hours from the time of injection the congestion began gradually to subside.

Its duration was variable. In three animals it lasted three days, in two animals four days, in two animals six days.

On examination of the charts one sees that the temperatures of the animals were elevated on the day of injection, and in one rabbit slightly elevated on the second day. On the third day the temperatures were normal. Thus the local congestion of the tuberculous area lasted considerably longer than the disturbance in temperature.

As the local reaction comes on, dilatation of the blood vessels leading to the tuberculous foci is first observed, and gradually increases in intensity. The cornea then becomes cloudy, and at times may become opaque. The conjunctiva becomes acutely inflamed and serum collects in the anterior chamber.

Of the constitutional disturbances, special attention was given to two: (1) Temperature, (2) Blood changes.

Temperature: On the evening of the day of injection the temperatures of the rabbits treated with tuberculin varied from 104° F. to 104.8° F. On the evening of the second day all the rabbits except one had a normal temperature. That rabbit had 103.6° on the second day, and had a local reaction which lasted six days.

Blood changes: During the reaction definite changes occurred not only in the number of leucocytes, but also in their relative proportions.

Daily for a few days previous to the injection, the blood of the animal while fasting was taken at the same hour. Blood counts were also made on healthy rabbits to establish, ap-

proximately, the normal number of leucocytes for a healthy rabbit in this altitude (1,500 feet). The counts were made on rabbits that had fasted for twenty-four hours, and varied from seventy-five to eighty-five hundred to the cubic millimeter. Lowit² found the leucocytes in the ear vein of healthy rabbits usually more than ten thousand to the cubic millimeter. In one case they reached sixteen thousand four hundred and twelve. As a rule he found them to fluctuate between ten and thirteen thousand. Estimations under ten thousand rarely occurred.

The tables of Brinckerhoff and Tyzzer³ show their lowest count for healthy rabbits to be six thousand four hundred and highest thirteen thousand four hundred. Their average in a large number of counts is nine thousand five hundred.

According to these last observers the average of a large number of differential counts of the blood of normal rabbits gives the following percentage of the leucocyte forms:

Lymphocytes	45	to	55	per cent
Large mononuclears	2	"	8	"
Amphophiles	40	"	50	"
Eosinophiles	0.5	"	1	"
Mast cells	4	"	8	"

It is worth mentioning that the amphophiles correspond to the neutrophile leucocytes of human blood. For a full description of the circulating blood of rabbits one is referred to the article of Brinckerhoff and Tyzzer.

The first change noticed and which began almost immediately after the injection was a diminution in the number of leucocytes (leucopenia) and it invariably occurred in both normal and tuberculous animals. It lasted sometimes four hours or longer. In one of the tuberculous animals the leucocytes dropped in four hours from six thousand to seventeen hundred and fourteen. Leucocytosis then followed. It began at a variable time, usually about four hours after the injections, though on several occasions it was well marked within four hours. In one instance it lasted till the fourth day, in

several till the third day. In some instances it ceased in twenty-four hours.

DIFFERENTIAL COUNTS. — The changes which constantly occurred in the leucocytes at the height of the leucocytosis were an increase in the amphophiles and a decrease in the lymphocytes. These changes were usually present within four hours from the time of the injection. In several experiments, during the leucocytosis, the amphophiles increased by forty per cent, and the lymphocytes decreased correspondingly. Up to thirty-six hours from the time of the injection the amphophiles were still in the majority. On the third and fourth days the amphophiles were in the minority, and the lymphocytes had correspondingly increased. The leucocytes then gradually returned to their normal relative proportions.

THE CHANGES THAT FOLLOWED THE INJECTION OF TUBERCULIN MODIFIED BY PEPSIN. — This was Koch's old tuberculin digested in the incubator at 37° C. with pepsin for seven to fourteen days. Two experiments were made. In both the rabbits there was well marked but delayed local reaction. Congestion of the eye was first noticed in from twelve to fourteen hours after the injection, and was but slightly developed. In one case it lasted twenty-four hours, and in the other forty-eight hours. In both rabbits there was no disturbance in temperature following the injections.

Precisely the same changes were observed in the blood of these two animals as has been described in the blood of the rabbits inoculated with tuberculin.

THE CHANGES THAT FOLLOWED THE INJECTION OF TUBERCULIN MODIFIED BY TRYPSIN. — This was prepared by digesting tuberculin with trypsin for seven to fourteen days in the incubator at 37° C.

Eight experiments were made. There was a marked fever reaction in but one rabbit, a slight fever reaction in one, and no fever reaction in the other six rabbits. There were no local reactions in the eyes of any of the rabbits injected.

In the two rabbits which had a fever reaction there were observed the same changes in the blood as have been described as occurring in the blood of the rabbits injected with tuberculin, or tuberculin modified by pepsin. In the rabbits that had no fever and no local reaction, there was no change in the blood.

CONCLUSION. — 1. With Koch's old tuberculin the changes that occurred during the reaction were, (1) fever, (2) local reaction, (3) leucocytosis.

2. With tuberculin modified by pepsin, there was one fact common to the rabbits injected, viz., absence of fever.

There were thus: (1) absence of fever, (2) the presence of a local reaction, (3) the presence of leucocytosis.

(N. B. The experiments with pepsin digested tuberculin were only two in number and should be repeated.)

3. With tuberculin modified by trypsin the fact common to all the rabbits was absence of local reaction. The other changes varied according to the length of time the tuberculin was digested. In the experiments in which it was digested for seven days, there was, (1) no local reaction, (2) the presence of fever, (3) the presence of leucocytosis.

In those experiments in which it was digested fourteen days there was, (1) no local reaction, (2) no fever, (3) no leucocytosis.

4. Leucocytosis and increase in the amphophiles with corresponding decrease in the lymphocytes always occurred together.

(I wish to express my thanks to Drs. E. L. Trudeau, E. R. Baldwin, and P. A. Levene for the kind interest they took in this investigation.)

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No. 15 RABBIT. (*Pipist.*)

DATE 1901.	CONDITION OF THE EYE.	SUBSTANCE INJECTED AND TIME OF INJECTION.	TEMPERATURE AT 5 P.M.	CONDITION OF THE EYE AFTER INJECTION.	LEUCOCYTES.	MONONUCLEARS.				ESTIMATED AMPLITUDE OF PHILIP'S.
						Lymphocytes.	Large mono-nuclears.	Total.		
								Per cent.	Per cent.	
Feb. 28.	Exophthalmos of right eye. Dimness of cornea and cheesy masses scattered over the iris.				5,555	40	21½	61½	38½	
March 1.			101.1° F.		6,222	58½	4	62½	37½	
" 2.		Given at 8 A.M. 2 c.c. of solution = 2½ c.c. old tuberculin.	104° F.	Reaction. — Hyperæmia and opacity of cornea (delayed reaction).	5,333 (count taken 4 hrs. after injection.)	50	1	51	49	
" 3.			100.6° F.	Marked local reaction.	8,000	50½	6	56½	43½	
" 4.			100.3° F.	Reaction still present but less intense.	8,923	62½	1½	64	36	
" 5.			99.8° F.	Reaction still slightly present.	8,769	42	6½	48½	51½	

No. 15 RABBIT. (*Pepsin.*) — *Continued.*

DATE 1901.	CONDITION OF THE EYE.	SUBSTANCE INJECTED AND TIME OF INJECTION.	TEMPERATURE AT 5 P.M.	CONDITION OF THE EYE AFTER INJECTION.	LEUCOCYTES.	MONONUCLEARS.			AMPHIPHILIC.
						Lymphocytes.	Large mono-nuclears.	Total.	
						Per cent.	Per cent.	Per cent.	Per cent.
March 6.			100.6° F.	Reaction has ceased. The cornea seems clearer than it was before the injection.	10,923	43	11	54	46
" 8.					7,666	30	21	51	48½
" 11.		Given at 8 A.M. 1 c.c. of pepsin-digested tuberculin = 1 c.c. Koch's old tuberculin.	103° F.	At 6 P.M. there was slight opacity of cornea, but no marked congestion of the eye.	4,615 (4 hrs. after injection.)	22	13	35	65
" 12.			102.6° F.	Reaction present, but not a strong one. Opacity and congestion of cornea.	11,692	40½	5	45½	54½
" 13.			102° F.	Reaction still present.	7,846	51½	5	56½	43½
" 14.			102.2° F.	Reaction ceased.	19,277	35½	5	40½	59½
" 15.			101° F.		7,384	41½			
" 16.					7,384		9½	59½	49½

" 18.	Given at 8 A.M. 1 c.c. old tuberculin.	104.8° F.	Reaction well marked 4 hours after injection. Cornea opaque and ves- sels congested.	7,500 (4 hrs. after injec- tion.)	35½	3	38½	61½
" 19.	101° F.	Strong reaction.	6,615	29	17½	46½	53½
" 20.	102.4° F.	Reaction still present, but subsiding.	12,923	52½	3½	56	44
" 21.	102.4° F.	Reaction ceased.	6,461	52½	11½	63½	36½

No. 12 RABBIT. (*Pepsin.*)

Feb. 28.	Dimness of cornea; large cheesy mass on iris at top of eye.	5,777	34½	10	44½	55½
March 1.	101° F.	8,666	66	5½	71½	28½
" 11.	Given at 8 A.M., 1 c.c. old tuberculin.	104° F.	Strong local reaction of eye.	1,714 (4 hrs. after injec- tion.)	64.3	11.3	75.6	24.2
" 12.	103.6° F.	Reaction.	7,000	21	5½	26½	73½
" 13.	102° F.	"	10,857	44½	11	55½	44½
" 14.	100.3° F.	"	9,692	62½	5½	68	32

No. 12 RABBIT. (*Pepsin*.) — *Continued*.

DATE 1901.	CONDITION OF THE EYE.	SUBSTANCE INJECTED AND TIME OF INJECTION.	TEMPERATURE AT 5 P.M.	CONDITION OF THE EYE AFTER INJECTION.	LEUCOCYTES.	MONONUCLEARS.			AMPHOPHILERS.
						Lymphocytes.	Large mononuclears.	Total.	
						Per cent.	Per cent.	Per cent.	Per cent.
March 15.									
" 16.			101° F.	Reaction.	8,307	69½	2½	72½	27½
" 18.			Reaction present, but has almost ceased.	11,076	58½	2½	61	39
" 19.		Given at 8 A.M., 1 c.c. pepsin-digested tuberculin = 1 c.c. old tuberculin.	100.8° F.	No reaction at 6 P.M.	4,923	19½	5	24½	75½
" 20.			102° F.	Slight reaction.	7,000	27	1	28	72
" 21.			101.4° F.	Reaction ceased.	4,923	56½	1½	57½	42½
" 21.			102° F.	" "	7,285	36½	1½	38	62

RABBIT FROM CAGE A. EXPERIMENT 153. (*Trypsin*.)

April 1.	Cornea opaque with large cheesy masses over it.	102° F.	13,529	58	3½	61½	38½
" 2.	102° F.	8,461	40½	7	47½	52½
" 3.	Given at 8 A.M., 1 c.c. trypsin-digested tu- berculin = 1 c.c. Koch's old tubercu- lin.	104° F.	No local reaction.	10,727	32	10	42	58
" 4.	102.6° F.	" "	15,692	40½	2½	43	57
" 5.	103.4° F.	" "	0	0	0	0	0
" 6.	102° F.	" "	7,230	44½	25½	70	30
" 8.	Given at 8 A.M., 1 c.c. Koch's old tubercu- lin.	104.4° F.	Strong local reaction.	5,846	17½	9½	27	73
" 9.	102.6° F.	" "	19,333	20	28½	48½	51½
" 10.	102.4° F.	" "	No count.	0	0	0	0
" 11.	103.2° F.	Reaction has ceased.	9,000	72½	6½	78½	21½
" 12.	103° F.	No count.				
" 13.	0	11,166	74	7½	81½	18½

No. 12 RABBIT. (*Pepsin.*) — *Continued.*

DATE 1901.	CONDITION OF THE EYE.	SUBSTANCE INJECTED AND TIME OF INJECTION.	TEMPERATURE AT 5 P.M.	CONDITION OF THE EYE AFTER INJECTION.	LEUCOCYTES.	MONONUCLEARS.			AMPHIPHILS.
						Lymphocytes.	Large mono-nuclears.	Total.	
						<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	
March 15.			101° F.	Reaction.	8,307	69½	2½	72½	27½
" 16.			Reaction present, but has almost ceased.	11,076	58½	2½	61	39
" 18.		Given at 8 A.M., 1 c.c. pepsin-digested tuberculin = 1 c.c. old tuberculin.	100.8° F.	No reaction at 6 P.M.	4,923	19½	5	24½	73½
" 19.			102° F.	Slight reaction.	7,000	27	1	28	72
" 20.			101.4° F.	Reaction ceased.	4,923	56½	1½	57½	42½
" 21.			102° F.	" "	7,285	36½	1½	38	62

RABBIT FROM CAGE A. EXPERIMENT 153. (*Trypsin*.)

April 1.	Cornea opaque with large cheesy masses over it.	102° F.	13,529	58	3½	61½	38½
" 2.	102° F.	8,461	40½	7	47½	52½
" 3.	Given at 8 A.M., 1 c.c. trypsin-digested tu- berculin = 1 c.c. Koch's old tubercu- lin.	104° F.	No local reaction.	10,727	32	10	42	58
" 4.	102.6° F.	" "	15,692	40½	2½	43	57
" 5.	103.4° F.	" "	0	0	0	0	0
" 6.	102° F.	" "	7,230	44½	25½	70	30
" 8.	Given at 8 A.M., 1 c.c. Koch's old tubercu- lin.	104.4° F.	Strong local reaction.	5,846	17½	9½	27	73
" 9.	102.6° F.	" "	19,333	20	28½	48½	51½
" 10.	102.4° F.	" "	No count.	0	0	0	0
" 11.	103.2° F.	Reaction has ceased.	9,000	72½	6½	78½	21½
" 12.	103° F.	No count.
" 13.	0	11,166	74	7½	81½	18½

RABBIT FROM CAGE G OF EXPERIMENT 153. (*Trypsin*.)

DATE 1901.	CONDITION OF THE EYE.	SUBSTANCE INJECTED AND TIME OF INJECTION.	TEMPERATURE AT 5 P.M.	CONDITION OF THE EYE AFTER INJECTION.	LEUCOCYTES.	MONONUCLEARS.			AMPHIPHILUS.
						Lymphocytes.	Large mononuclears.	Total.	
						Per cent.	Per cent.	Per cent.	Per cent.
April 1.	Dimness of cornea with large tubercles over its upper part.	100.6° F.	9,083	37	14	51	49
" 2.	100.8° F.	11,200	15	19	34	66
" 3.	Given at 8 A.M., $\frac{1}{2}$ c.c. trypsin-digested tuberculin = $\frac{1}{2}$ c.c. old tuberculin.	103° F.	No local reaction.	12,000	15	14 $\frac{1}{2}$	29 $\frac{1}{2}$	70 $\frac{1}{2}$
" 4.	102° F.	" " "	12,000	41 $\frac{1}{2}$	8 $\frac{1}{2}$	50	50
" 5.	102.2° F.	" " "	No count.				
" 6.	101.8° F.	" " "	11,818	53	8	61	39
" 8.	Given at 8 A.M., $\frac{1}{2}$ c.c. old tuberculin.	104.4° F.	Well marked local reaction.	8,571	15 $\frac{1}{2}$	8	23 $\frac{1}{2}$	76 $\frac{1}{2}$

" 9.	101° F.	Strong reaction.	13,142	9	38	62
" 10.	101.2° F.	Reaction present.	No count.			
" 11.	102.5° F.	Slight reaction.	6,923	No count		
" 12.	103° F.	Reaction has ceased.	0	"		
" 13.	0	11,833	28½	44½	55½

RABBIT No. 9. (*Trypsin*.)

1900. Dec. 11.	Small tubercles the size of a pin's head scattered over the iris.	5,400			
" 12.	101.1° F.				
" 13.	101° F.	10,800			
	Given at 8 A. M., 2 c.c. trypsin-digested tu- berculin = 240 mil- ligrammes old tu- berculin.	102° F.	No local reaction.	7,840			
" 14.	101° F.	" " "	6,360			
" 15.	102.2° F.	" " "	7,625			
" 16.	0	" " "	6,200			

RABBIT NO. 9. (*Trypsin.*) — *Continued.*

DATE 1900.	CONDITION OF THE EYE.	SUBSTANCE INJECTED AND TIME OF INJECTION.	TEMPERATURE AT 5 P.M.	CONDITION OF THE EYE AFTER INJECTION.	LEUCOCYTES.	MONONUCLEARS.			AMPHOPHILES.
						Lymphocytes.	Large mononuclears.	Total.	
Dec. 17.	Given at 8 A.M., 1 c.c. old tuberculin.	Not taken.	Marked local reaction.	14,570 (4 hrs. after injection.)				
" 18.	Not taken.	" "	4,600				
" 19.	" "	6,200				
" 20.	" "	5,800				
" 21.	100.4° F.	5,888				
" 22.	102.3° F.	Still slight reaction.	4,900				
" 23.	Reaction ceased.					

RABBIT No. 10. (*Trypsin*.)

Dec. 11.	Small tubercles about size of pin's head over iris.	102° F.	6,000		
" 12.	101.2° F.	10,600		
" 13.	Given at 12 noon, 2.40 milligrammes old tuberculin.	102° F.	Marked local reaction.	10,600 (5 hrs. after injection.)		
" 14.	101° F.	" "	8,500		
" 15.	101° F.	Slight local reaction.	4,800		
" 16.	Reaction ceased.			
" 17.	Given at 12.45 P.M., 240 milligrammes trypsin-digested tu- berculin.	No local reaction.	8,156 at 3.30 P.M.		
" 18.	No local reaction.	4,000		
" 19.	" "			

N.B.—The remaining trypsin tests were done conjointly with Drs. Baldwin and Levene, and appeared in their article. (Journal of Medical Research, Vol. VI., No. 1, page 129.)

A STUDY OF CHRONIC INFECTION AND SUBINFECTION BY
THE COLON BACILLUS.

I. ON THE ANEMIA PRODUCED BY REPEATED INJECTIONS
OF CULTURES OF A COLON BACILLUS OF LOW VIRU-
LENCE.

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That there is some relationship between morbid conditions in the alimentary canal and sundry forms of anemia, has been held for long years by many clinicians. Since the year 1850, indeed, it has been recognized that the presence of the *Anchylostoma* or *Dochmius*, or, as parasitologists now insist, the *Uncinaria Duodenalis*, is responsible for the marked anemia in subtropical regions; so common in Egypt as to have gained the name of Egyptian Chlorosis. A yet more intense anemia is brought about by the *Bothriocephalus latus*, an anemia closely resembling the typical pernicious anemia. Years ago Hay of Aberdeen regarded chlorosis as of digestive origin and treated it by means of large doses of magnesium sulphate. Similar circumstances led Sir Andrew Clarke to copy and extend Hay's treatment. Still more recently the studies of Hunter and other observers have led to an increased belief that pernicious anemia is secondary to chronic gastritis, either hypertrophic or atrophic.

In the case of the anemias induced by intestinal parasites the older opinion has been, that they are due to the abstraction and leakage of blood brought about by the worms and their bites. Now several workers in Italy and elsewhere are bringing forward evidences that toxic substances, elaborated by the parasites, are the direct cause. In chlorosis the view held has been that with retention of fecal matter, products of bacterial fermentation undergoing absorption in increased amounts exert hemolytic action in the blood. In pernicious

anemia, Hunter¹ has suggested a similar absorption, more especially from the stomach and upper part of the digestive tract.

So long as it was accepted on all sides that the intact mucosæ did not permit the passage of bacteria, for so long was it impossible to advance much further, though curiously, to the best of my knowledge, until within the last few months no one has attempted to investigate the effects upon the blood of repeated injections of bacteria or of their toxines, or of one or other product of bacterial fermentation.

But if, as several observers have indicated,² and as Ford³ would seem to have proved beyond the possibility of a doubt, bacteria normally are taken up in small numbers from the digestive canal and are to be found in and cultivated from the liver and other organs, another possibility presents itself. It is possible that low inflammatory states of the intestinal mucosa, coupled with more or less retention of the contents of the alimentary canal, may be accompanied not merely by an increased absorption of products of fermentation, but also by an increased taking up of the bacteria from the intestinal contents — of bacteria which are not necessarily of a high virulence, which are constantly being destroyed in one or other organ; and it may be assumed that such bacteria gaining entrance, in increased numbers, into the blood and lymph, if they do not set up a frank infection will ultimately by their products have a deleterious influence upon the tissues and fluids of the organism leading to a condition which Professor Adami would term "Subinfection." It is at least possible, therefore, that some of the blood changes in certain forms of anemia are indications of such a condition of subinfection.

Now these two possible factors in the production of anemic states — the action of absorbed bacterial toxines and that of the constant presence of bacteria of low virulence in the circulating blood — deserve to be tested, nor is it difficult to devise means whereby this can be accomplished. The main difficulty in carrying out studies of this order is that the individual experiments require a routine treatment and routine

study of the blood and general conditions of the animals selected for the study, over relatively long periods of time.

The form of micro-organism to be employed for these observations immediately suggests itself, namely, the Colon bacillus, the commonest inhabitant of the intestinal tract — a form which under normal conditions is of so low a virulence that growing and multiplying in the intestines it sets up no disturbance whatsoever. We know, however, that it is far from being devoid of virulence, and that it is capable under certain conditions of gaining increased powers, of invading the organism, and of manifesting very definite pathogenic effects.

To my knowledge, observations of this nature have not hitherto been attempted. Ten years ago Blachstein,⁴ working under Welch, pointed out very clearly the different results to be obtained by employing colon bacilli of different degrees of virulence, and varying the amounts of cultures inoculated. He showed that there might be produced an acute disease leading to death within thirty-six hours, a subacute disease with death occurring at the end of from three to twenty days, and a more chronic condition in which after the preliminary disturbance set up by the inoculation, the animal appeared to be unaffected for a fortnight or three weeks, then progressive emaciation became noticeable leading to death at the end of a month or six weeks. In this Laboratory we have repeatedly confirmed these observations of Blachstein — but neither in Baltimore, here, nor elsewhere, have they been greatly extended, nor have studies been made upon the state of the blood in this more chronic infection. Still less have any observations been made upon the effects of repeated inoculations of smaller quantities of pure cultures of *B. coli* or of what I may term sublethal cultures, *i.e.*, cultures which under ordinary conditions produce no signs of progressive infection after a single inoculation.

Thus the task which I have set myself has been to obtain cultures of a typical colon bacillus from the healthy intestine and to study the effects of (1) the repeated injection of small amounts of pure culture of the same into the organism, (2)

the repeated injection of the fluid cultures with the dead bacilli, and (3) the effects produced by the toxins; thus to determine if anemia be producible, and if so, of what order. As above indicated, observations of this nature demand the careful study of each animal acted upon over a period of several weeks, not to say months; thus I have not nearly completed the whole series of investigations. In this communication I shall deal only with the results obtained by injections of the living bacteria, leaving to a later communication the details of the series of studies upon the periodic injection of toxins, etc., and a comparison of the results. While my attention has been more especially directed to the blood conditions, I shall have, now and later, to call attention to other morbid states resulting from this subinfection.

On the Selection of a Culture of B. Coli. — In selecting a strain of the colon bacillus for these experiments, two conditions had to be satisfied, (1) that the form selected should possess and retain typical reactions outside the body, and (2) that it should possess and retain a definite low grade of virulence. Not only is there a great number of species, or at least subspecies, included under the name Colon Bacillus, as Ford⁵ working in this Laboratory has more especially emphasized; but, as Durham⁶ has pointed out, the form which corresponds with the original description of *B. coli communis* is not the most common; we have, that is, to recognize *B. coli communis* (Escherich), which ferments dextrose and lactose, but does not ferment saccharose, and *B. communior* fermenting all three sugars, and this last, being the most common, has been taken as the type of the group. It is more than likely that forms manifesting constant slight differences in reaction towards different nutrient media manifest constant slight differences in pathogenic properties; in fact, studying the scale of closely allied forms from the colon proper through the Shiga bacillus to the Gärtner group and *B. typhosus*, we know that this is the case. It is of prime importance, therefore, to select a definite type form to employ all through such a series of observations;

indeed, neglect to recognize these constant pathogenic differences between different strains of the colon bacillus is certainly responsible for divergent statements by previous workers on colon infection.

It may well be that others of the forms grouped under the general title of Colon bacilli produce a different grade or type of anemia; indeed, it is deserving of note that from the stomach and intestines of cases of pernicious anemia, the form isolated by Anderson, myself, and other workers in this Laboratory, has so far not been *B. coli communis* of Escherich, nor *B. communior* of Durham, but that designated by Ford, *B. coli* "E," fermenting dextrose and saccharose, but not lactose. It will be well to study these other forms and their effects later; in the meantime it has seemed proper to select for this first study the most widely distributed form; thus, therefore, I have employed a strain of the *B. coli communior*.

In the second place, regarding the virulence, it appeared important to obtain the micro-organism from the intestinal canal of sound healthy animals of the same species as those to be employed later in making the investigations. What was needed for these experiments was a form which would not set up a powerful infection; the usual relatively harmless symbiotic bacillus of the intestine appeared likely to satisfy this condition. It seemed possible that a colon bacillus grown for a considerable period in, *e.g.*, the human intestine, might gain or lose in virulence and all that is included under that term, when transferred to animals of another species; it was safer not to introduce any possible disturbing factor of this nature. Hence as rabbits were indicated as the most satisfactory animals to employ for the purposes of this investigation, it was from a rabbit that I obtained my stock culture. I have since learned that I did well in making this decision, for in an article recently published ("*American Medicine*," March 29, 1902) Veranus A. Moore and Floyd R. Wright call attention to the fact that the colon bacillus is not very common in the intestine of the rabbit, occurring in only about one in four animals; yet all

the cultures of the colon bacillus isolated by them from the intestinal canal of the rabbit were of the commonest form, the *B. communior* of Durham. The characters of this colon bacillus are briefly as follows: It is motile and is less than one micromillimeter in diameter; does not form spores; grown in broth it produces turbidity and a pellicle in twenty-four hours;* it does not give a dull or wrinkled growth on agar, and fails to show a characteristic appearance on gelatin plates. It grows well on potato; does not liquefy gelatin, casein, or blood serum; ferments dextrose, lactose, and saccharose broth with the production of gas and acid; grows in the closed arm of the fermentation tube; coagulates milk with the production of acidity in the first twenty-four hours; produces nitrites, indol, and a fecal odor. It is a facultative anaerobe, growing best on neutral or slightly acid media, and is of a low degree of virulence.

Methods of Inoculation.—Having now in pure culture a form of the colon bacillus suitable for the purposes of these investigations, healthy adult rabbits were selected and divided into two groups: Those in Group I. were given periodic small doses of a twenty-four-hour broth culture of the colon bacillus injected into the abdominal cavity. Those in Group II. received smaller doses of a similar culture injected directly into the circulating blood through the ear vein. As a control, another rabbit was placed under similar routine observations and treatment, except that the injections consisted of sterile broth instead of the active cultures of *B. coli*. Strict antiseptic precautions were observed in administering the injections and throughout the experiment; both with regard to the cultures of *B. coli*, and also the rabbits, every precaution was taken to exclude the presence of adventitious bacteria.

* While correcting the proof of this article my attention has been called to a statement by Gage (Bacteriological Studies at the Lawrence Experiment Station with special reference to the determination of *B. Coli*, 33rd Ann. Rep. State Board of Health of Massachusetts for 1901), to the effect that "*B. Coli* should never form a pellicle in fluid media." (Reprint, p. 5.) This is directly contrary to our experience. A pellicle may or may not be formed on neutral broth according to the strain or member of the group employed.

In the routine observations upon the general condition of the rabbits, daily note was taken of the weight of each rabbit every morning before the animals were fed, and daily, at the same hour, morning and evening, the body temperature of each rabbit was noted. A routine examination of the blood was made every third day; the total number of red and white corpuscles was ascertained by means of the Thoma-Zeiss Hemocytometer, and the percentage of hemoglobin was estimated by the Oliver method. Dried films of the blood were prepared and stained by various methods. That which gave the most satisfactory results was Wright's⁷ modification of Leischman's (Romanowsky) method.

The Injection of an Active Culture of the Colon Bacillus. — Blachstein, and various workers in this Laboratory, have noted that there is considerable variation of susceptibility among rabbits towards injections of cultures of the colon bacillus. This, too, has been my experience. Beginning with small doses — 0.25 cc. injected into the ear vein, and 1.0 cc. injected into the abdominal cavity — I have been able to increase the amount considerably in some cases. The amount of the dose was regulated, by the general condition of the animal and its susceptibility, in such a manner that each rabbit received a trifle more of the culture each time than its minimum dose, thus producing and maintaining a state of chronic subinfection which in time wrought grave changes in the blood and tissues of the animals.

The immediate effect of an injection of the active culture was a period of dulness and langor, which, however, soon passed off. The temperature rose one to three degrees Fahrenheit, the maximum being reached in four to six hours, when a gradual decline followed, so that after twenty-four to thirty-six hours the temperature had again become normal. At first it was usual to allow four days to intervene between the injections of the culture, but after a few weeks it was possible to gradually shorten this interval to two days, thus maintaining with certainty and regularity the condition of subinfection aimed at. The effects were soon manifested. The

weight of all the animals began to decrease. At first the rate of decrease was about equal in both groups; later, however, it became slower in Group I., and finally gave place to a slight gain; while in Group II. the decrease continued at about the same rate throughout the experiment, although at no time was the appetite of the rabbits affected, for they consumed proportionately as much food as the control rabbit, whose weight varied but little one way or the other.

Not long after the experiment had begun certain changes in the blood were apparent. The number of red cells began to decrease so that at the end of the first month the total number per cubic millimeter, which at the beginning of the experiment had ranged between 5,500,000 and 5,750,000, fell to between 3,000,000 and 5,000,000, a loss of 2,250,000 in the one case, and of 750,000 in the other. The percentage of hemoglobin likewise decreased to between fifteen and twenty-five per cent. There was slight leucocytosis, in fact at no time did any of the rabbits exhibit a marked rise in the number of white cells. The leucocytosis was chiefly due to an increase in the number of polymorphonuclear leucocytes and the eosinophiles. At this period a slight degree of poikilocytosis was noticed in the red cells of the rabbit whose blood count had fallen to 3,000,000; afterwards it was observed that it was at about this point, namely, when the red corpuscles had become reduced to about 3,000,000 per cubic millimeter, that poikilocytosis first appeared in other rabbits of the series.

During the second month the decrease in the number of the red cells, in the case of the rabbits injected intraperitoneally, was not so rapid as during the first month, amounting only to 250,000. It was different, however, with the rabbits of the other group (receiving an intravenous inoculation); in their case the loss in red corpuscles continued so that the total number at the end of the month ranged between 2,500,000 and 3,000,000. Each rabbit in this group now showed well-marked poikilocytosis of the red cells; pear-shaped cells, crescents, macrocytes, and microcytes were common; and in the stained films from the rabbit with the lowest count, nucleated red cells could readily be found.

In this rabbit, besides these interesting changes noted in the blood, certain effects upon the nervous system began to manifest themselves, first in the lower lumbar neurones causing incoördination and loss of function of the hind legs. The reflexes were increased and clonus could readily be demonstrated. The rabbit hobbled about with difficulty, but was not otherwise inconvenienced. To what extent these nerve changes might have progressed could not at that time be surmised, and as a well-marked anemia had been produced in all the rabbits I decided to test the recuperative powers of the animals and ascertain to what extent this anemia could be recovered from in a given time; and by continuing to subject the animals to the same conditions, save that of injecting the cultures of the colon bacillus, it appeared to me that it would be possible to find out if any other factor was at work in the production of the anemia. Therefore the injections of the *B. coli* were discontinued.

For a few days the number of red corpuscles continued to decrease, then a reaction set in and there was a rapid return to the normal. The number of red corpuscles steadily increased and rose from 2,500,000 — the lowest point to which any one of the rabbits had been reduced — to 4,500,000 in the same rabbit, a gain of 2,000,000. The poikilocytosis entirely disappeared, so that the red cells were once more of the normal size and shape. Nucleated red cells could no longer be found. The percentage of hemoglobin rose with the increase in the number of red cells and reached the normal. The number of leucocytes, though at no time markedly high, decreased somewhat. There was also some gain in weight. Those rabbits which had exhibited certain effects upon the nervous system improved rapidly, and although they did not recover completely, the symptoms were much ameliorated. In short, at the end of the two weeks' "rest" all the rabbits had improved to such an extent in general condition as to justify the opinion that in a short time all traces of the previous anemia would disappear. It was now quite evident that the injections of the colon bacillus had had much, if not all, to do with the causation of the

anemia, and unless a sufficient degree of immunity had been acquired, it was probable that the anemia would return as soon as the injections of the *B. coli* cultures were resumed; that is, a relapse would follow a return to the condition of subinfection.

The injections of the *B. coli* cultures were therefore resumed, taking care at first that the dose was not too large. This time the rabbits were more susceptible than at first, so that not many days elapsed before the former symptoms began to return. The red corpuscles were reduced in number even more rapidly than formerly. Poikilocytosis returned and nucleated red cells now became fairly common in the blood of all the animals. The hemoglobin percentages decreased *pari passu* with the number of red corpuscles in the blood. The number of leucocytes did not increase as formerly, but continued to slowly decrease until the total number was as low, or in some cases lower, than at the beginning of the experiment. The nervous system was again attacked, this time with greater severity than at first, all the limbs being affected to a greater or less degree. In some cases there was marked wasting of the muscles as well as loss of power. The reflexes, at first exaggerated, were later on entirely lost, and in two of the rabbits there was loss of control over the sphincters. In both of these rabbits the paralysis of the muscles of the limbs was complete several days before death, while the trunk muscles were very greatly affected during the last thirty-six hours of life. This extensive paralytic condition was accompanied by a remarkable fall in temperature. The average normal temperature of these rabbits was 102° F., after the onset of the progressive nerve changes it rose to 104° F., and at times to 105° F., but in the later stages, above referred to, it fell to 96° F. and even to 95° F. during the last twenty-four hours of life. In the last thirty-six hours before death not only were the sphincters paralyzed, but the animals were unable to swallow.

We have already called attention to the fact that the rabbits appeared to be more susceptible to the injection of the cultures during the "relapse" than during the first part of

the experiment, consequently it was possible to reach a higher degree of anemia.

Of the rabbits in Group I. injected intraperitoneally with the culture of *B. coli*, the red blood cells of No. 1 were reduced to 1,500,000, or 27.5 per cent of the original number. The percentage of hemoglobin fell to fifteen. Rabbit No. 2 of this group had died of acute peritonitis during the fifth week of the experiment, while rabbit No. 3 had died of acute toxemia within twenty-four hours after the first injection of the *B. coli* culture.

Of the three rabbits in Group II., which were injected in the ear vein, the red blood cells of No. 1 were reduced to 1,425,000, or 25.2 per cent of the total number present at the beginning of the experiment. The percentage of hemoglobin fell to ten.

In the case of rabbit No. 2 the red cells were reduced to 1,425,000, or 28.2 per cent of the normal number. The percentage of hemoglobin was ten.

At the same date as that of the last "count" in No. 2, rabbit No. 3 — the sole survivor of the group, and still alive at time of writing — showed a total of 1,750,000, or 33.3 per cent of the normal number. The percentage of hemoglobin had been reduced to thirty-five.

Thus the intravenous method appears to be the safer of the two and also gives the best results. Let me here note that the injections of the colon cultures were continued over several months; in fact, one member of Group I., which did not succumb to acute disease, was under observation and treatment for one hundred and fifty-nine days. Two members of Group II. lived for one hundred and seven and one hundred and fourteen days respectively, while the third member is still alive at the end of seven months from the beginning of the experiment. The control rabbit, treated with periodic injections of sterile broth, suffered no ill effects and was not affected in any manner by the treatment.

Concerning the rabbits which died of acute intoxication, nothing need be said, as I have nothing new to add to the extensive literature on the subject. The protocols of the

rabbits that died after being subjected for many weeks to repeated injections with the colon bacillus are as follows:

Group I., rabbit No. 1, weight 1,005 grams, treated with periodic inoculations of a twenty-four-hour broth culture of *B. coli communior*, injected into the abdominal cavity. Died 159th day, weight 925 grams. Chart No. 1.

Autopsy, immediately after death: The animal was in fairly good condition, some subcutaneous fat on flanks and on lower part of abdomen; muscles pale and soft, no free fluid in abdominal cavity. The intestines did not exhibit any evidence of acute or chronic inflammation, but were pale and anemic, otherwise normal. The bladder was distended with dark-colored urine, free from sugar and albumin. Upon emptying the bladder, a small well-formed calculus the size of a large grape seed was found. The kidneys were pale and anemic, but of normal size.

Liver slightly enlarged, nutmeg appearance, containing numerous small masses of coccidia. The gall bladder full of thin, pale reddish bile. The spleen was shrunken and ashen-gray in color.

The lungs were pale and anemic, otherwise normal. Heart somewhat enlarged, right side filled with thin blood.

Histologically: The liver presents a slight increase in fibrous tissue, but this may be due to the presence of coccidiosis. The liver cells show a slight degree of cloudy swelling. In many of the cells the nuclei are swollen and vacuolated and masses of pigment are very numerous in nearly all of the cells. Some of these masses took on the stain for iron, but on the whole the reaction of the sections to Perl's test was not characteristic. There was some proliferation of the endothelial cells of the bile capillaries.

In the kidneys some of the convoluted tubules showed proliferation of the lining cells, otherwise there was little departure from the normal.

The bone marrow was not markedly altered. There was some hyperplasia with a diminution of fat and an increase of lymphoid tissue.

Cultures were taken from the heart blood, bile, urine,

liver and kidney juices, and *B. coli communior* found to be present in all of them, being in pure culture in the heart blood, bile, and urine.

Of the rabbits in Group II. which had received the inoculations of broth cultures of colon bacillus injected into the ear vein, two have died. The animals were more emaciated than those in Group I., but in other respects the post mortem findings did not differ to any extent.

Group II., No. 1, weight 1,325 grams, treated with periodic inoculations into the ear vein, died 107th day, weight 815 grams. Loss, 510 grams, 38.49 per cent. Chart No. 2.

Autopsy: Emaciation extreme, inner surface of skin of a tawny-yellow color; muscles much wasted, pale, and dry. Peritoneum and intestines normal. Bladder distended, containing turbid urine, free from albumin; kidneys of normal size and appearance.

Liver of a dark earthy color, slightly smaller than normal. Gall bladder filled with thin reddish bile. Spleen shrunken and of a dark gray color. Lymphatic glands not palpable.

Lungs normal. Heart somewhat dilated, right side distended with blood, left side contracted.

Cultures were taken from the heart blood, bile, liver and kidney juices, and urine. Those taken from the liver and kidney were sterile, but pure cultures of the *B. coli communior* were obtained from the heart blood, bile, and urine.

Histologically: Sections from the liver were free from fibrosis, but the cells in some areas showed cloudy swelling. The nuclei of a large part of the liver cells were swollen and vacuolated. The liver cells contained much pigment which in some places took the stain for iron.

Heart: In some parts the muscle fibers were swollen and had lost their striation, in other areas definite fatty degeneration could be made out.

The kidneys were somewhat congested and showed slight cloudy swelling, but otherwise were normal.

Rabbit No. 2: Weight 1,380 grams. Died 114th day, weight 665 grams, loss 715 grams, or 51.8 per cent. Chart 3.

Autopsy: Extreme emaciation; muscles wasted, pale, and

dry. Bladder empty. The abdominal viscera were entirely free from signs of peritonitis. The various organs showed the same conditions as those found in No. 1.

Microscopically, the heart muscle fibers showed cloudy swelling with beginning fatty degeneration.

Liver: There is cloudy swelling present generally, while a few small areas show beginning necrosis. The liver cells are loaded with pigment and many of them contain coccoid and diplococcoid forms.

The kidneys are considerably congested, but otherwise normal. As before, pure cultures of *B. coli* were obtained from the heart blood, bile, and urine.

These rabbits were not ill at any time and apparently died from exhaustion. The histological changes found in the spinal cord will be reported in a future communication. At present I will state that diffuse degeneration has taken place in the columns of Gall and in the lateral areas of the cord, closely resembling the conditions described as occurring in the spinal cord in certain cases of pernicious anemia.

SUMMARY.

The above observations upon the two sets of rabbits, one of which received small doses of cultures of a non-virulent form of *B. coli* injected into the abdominal cavity, while the other received smaller amounts of the same culture intravenously, give such concordant results—and other rabbits not here mentioned treated in a slightly different manner are now giving me like results—that we may safely regard the findings here described as characteristic and of general application.

It may therefore be laid down that “subinfection” of rabbits with *B. coli* leads to the following results:

1. A MARKED AND PROGRESSIVE ANEMIA having the following features:

(a.) Reduction in the number of red corpuscles, which may attain so extreme a degree that in place of the normal

five and a half millions or more, there may be only one and a half millions — a reduction, that is, of seventy-four per cent.

(*b.*) Poikilocytosis: This shows itself when the erythrocytes have sunk to about 3,000,000. All the forms noted in poikilocytosis in man are here observed, namely, pear-shaped cells, crescentic cells, macrocytes and microcytes.

(*c.*) Appearance of nucleated red corpuscles: This was not so marked in the first period of the observations, but was relatively common in the second part, or period of relapse.

(*d.*) The hemoglobin in general sinks progressively with the decline in number of the red corpuscles, although some variation is observable; rabbit No. 3 of group II. showing a relatively high percentage of hemoglobin, *i.e.*,

	Lowest Number of Red Corpuscles.	Hemoglobin Percentage at the same Period.
Group I., Rabbit No. 1,	1,500,000	15
“ II., “ “ 1,	1,425,000	10
“ II., “ “ 2,	1,425,000	10
“ II., “ “ 3,	1,750,000	35

(*e.*) The alteration in the number of leucocytes is but little marked. There was observed at first a slight increase from the normal 2,000 or 2,500 up to 3,000 and 8,000 — the highest leucocytosis being in the case of the rabbit receiving the intraperitoneal injections. In the second period this leucocytosis scarcely showed itself; indeed, the number of leucocytes in general became reduced to below the normal.

(*f.*) Accompanying the anemia there was no loss of appetite, but in the two series of rabbits a slight difference was observable as regards the weight and general conditions. Those animals receiving an intravenous inoculation underwent a progressive emaciation and loss of weight, whereas the one animal which withstood the intraperitoneal injections showed a less marked loss of weight during the first part of the observations, and gained appreciably in weight during

the interval, and this weight was sustained during the second part until the end.

				Loss of weight end of 1st period. <i>Grams.</i>	Gain during interval. <i>Grams.</i>	Loss during 2d period. <i>Grams.</i>
Group I.,	Rabbit 1	.	.	100	53	27.5
" II.,	" 1	.	.	409	103	433
" II.,	" 2	.	.	200	50	275
" II.,	" 3	.	.	100	45	120

The one group of animals showed no subcutaneous fat at autopsy, but this was still present in fair amount in the other case.

2. PROGRESSIVE EFFECTS UPON THE NERVOUS SYSTEM.

—All the animals developed a condition of progressive, ascending paresis, giving place to paralysis with the development of exaggerated reflexes in the earlier period, followed by complete loss. Eventually the sphincters became paralyzed and shortly before death the muscles of deglutition appeared to be involved. Throughout the experiment no convulsive attacks or seizures manifested themselves. There was no evidence of arthritis, nor was there at any time any tenderness of the joints. For a while during the period of exaggerated reflexes, tactile hyperesthesia could be made out over the posterior half of the body.

3. OTHER SYSTEMS. — The Circulatory system presented no changes in the arteries. The heart muscle showed some loss of striation and cloudy changes, and in one instance definite fatty change could be made out.

The Respiratory system was negative. In the Lymphatic system the glands showed no enlargement. The spleens in all cases were either normal in size or definitely shrunken. The Bone-marrow showed at most a diminution in fat with some hyperplasia and an increase of lymphoid tissue.

The Livers showed no cirrhotic change other than that which might be explained by the simultaneous existence of old coccidiosis. Perls' test showed that there was some in-

crease in the amount of iron deposited in the liver cells, but this was not pronounced.

There was no diarrhea nor distinct evidence of disturbance of the Digestive system until the sphincters became involved.

CONCLUSIONS.

From the above epitome of the conditions found it will be observed that in these experiments there has been developed a very remarkable state of advanced anemia. That anemia is not quite comparable with any of the classic forms seen in man. In some respects it is strikingly like the condition of Pernicious Anemia, namely, in the very great diminution in the number of erythrocytes, the marked poikilocytosis, and the appearance of nucleated red corpuscles.

As far as I know at the present time no other observer has produced these striking conditions. But, on the other hand, it differs from Pernicious Anemia in the fall of the amount of hemoglobin being parallel with the decrease of the red corpuscles; in the absence of a distinct Quincke's siderosis, or increased presence of iron in the livers; in the absence of any clear evidence of inflammatory or other disturbances of the digestive tract, and of well-marked changes in the bone-marrow.

Whether employment of other strains of the colon bacillus would lead to a picture more clearly resembling Pernicious Anemia, or whether again the employment of bacterial toxins rather than the pure attenuated cultures, will give different results, must be left for future studies. In the meantime I am inclined to think that the observations here recorded are of a certain value, as indicating one method, namely, that of "Subinfection" by the ordinary bacterium of the digestive tract, whereby a very definite grade of anemia may be produced.

(I desire to express my thanks to Professor Adami for many suggestions which have been of the greatest assistance in conducting these experiments.)

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ON THE HEMOLYTIC POTENCY OF CERTAIN SAPOTOXINS
DISSOLVED IN BLOOD-SERUM.

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The inhibitory action of blood serum on hemolysis by glucosides and glucosidal alkaloids, has been variously interpreted.

Hédon,¹ working with solanin, comes to the conclusion that the salts of serum favor hemolysis, while its proteids retard or hinder hemolysis. In support of his contention, Hédon states that serum, deprived of its salts by dialysis, and rendered isotonic by the addition of sodium chloride, inhibits the solvent action of solanin on the erythrocytes, more effectually than does normal serum.

Pohl,² on the other hand, asserts that acid sodium phosphate, by hindering the entrance of solanin into the corpuscles, greatly retards hemolysis, and that serum containing much of the salt is in a manner protective against solanin. According to him, therefore, acid sodium phosphate is the antibody to which serum owes its protective action against the alkaloid.

Ransom³ declares that the cholesterin of the plasma and corpuscles acts as an antidote against members of the saponin group, and that the addition of cholesterin to a saponin in solution causes the glucoside to lose its hemolytic action.

In view of Ransom's bold assertion, and of the contradictory statements made by Hédon and Pohl, in interpreting the restraining action of blood-serum on hemolysis by solanin, I thought it desirable to test the "protective" action of serum, using the more energetic sapotoxins as hemolytic agents.

Measured volumes of freshly shed blood were treated, in vitro, with solutions in blood-serum of quillaja-sapotoxin,

cyclamin, and saporubrin, of known strength, and the time necessary in order to complete laking was noted. The smallest amount of each sapotoxin capable, when dissolved in serum, of causing complete laking of a certain volume of blood was determined, and its laking action compared with that of an aqueous solution of the same strength.

Furthermore, known quantities of various salts, capable of affecting the permeability of the erythrocytes to the entrance of the sapotoxins, were dissolved in serum solutions of quillaja-sapotoxin and cyclamin, and the behavior of the blood, when subjected to the action of the salted solutions of these glucosides, was observed under the microscope, and with the naked eye.

In the preparation of the sapotoxin solutions, one was careful to use sterile serum. To this end, blood was received from a cut vein into a sterilized flask, with the care needed to ensure freedom from bacterial contamination, and then set aside in a cool place to coagulate. Within thirty-six hours, the serum, especially that from rabbit's blood, was found quite clear, and mostly free from dissolved hemoglobin.

The specific gravity of man's serum varied from 1.0293 to 1.03; that of rabbit's serum from 1.0247 to 1.0285, at 15° Celsius, as determined by Hammerschlag's method, the Westphal balance being used to ascertain the specific gravity of the benzol-chloroform mixture.

Samples of blood when treated with their corresponding sera (*e.g.*, rabbit's blood with rabbit's serum) gave no evidence of hemolysis, the red cells remaining intact as long as examined.

Care was taken in the selection of the sapotoxins employed, especially as regards their purity; for the researches of Christophson⁴ have shown that sapotoxins, from various sources, vary as to their toxicity and hemolytic potency, in proportion to their freedom from admixture with bodies formed, during their extraction. So that, in studying the hemolytic potency of divers sapotoxins, discrepancies in results are apt to occur, according as the glucosides are pure or crude.

TECHNIQUE.— The following procedure was adopted, in studying the action of serum, charged with quillaja-sapotoxin, cyclamin, and saporubrin, on the formed elements of the blood. Blood obtained from various sources, and contained in shallow cells, was irrigated with serum solutions of the poisons. The cell is made by affixing to a slide, previously cleansed, a clean, square cover-glass (seven-eighths inch), having two of its opposite edges smeared with a narrow and uniform layer of benzol-colophonium. The cover glass is applied with its painted edges facing downwards, and lying parallel to the long axis of the slide. By gentle pressure on the cover glass, until it is firmly fixed, the depth of the cell can be made as shallow as desired. Into such a cell blood flows readily by capillary attraction, and its features under normal and abnormal conditions, the ameboid movements of the larger leucocytes, and fibrin formation may be readily observed, under the microscope.

In order to study the action of the poisons on the dried erythrocytes, blood smears made upon slides, in the usual manner, were allowed to dry in air, and then fixed in hot air at 120° C. Then a cover glass, prepared as before, was applied over each film, thus converting the part of the slide on which it rested into a shallow cell, containing a thin and uniform film of dried blood.

Similarly, films fixed in other ways, by formaldehyde vapor, for instance, were covered over, leaving a capillary air space between the film and the cover glass.

Having charged the cell with blood, the film was examined microscopically, and its features noted.

When it was desired that the leucocytes of freshly drawn blood should be actively ameboid, that their behavior under the influence of the poisons might be studied, the cell-bearing slide was placed on a warm stage at about 30° C.

The blood contained in the capillary cell was readily irrigated, by placing a large drop of the sapotoxin solution at the entrance of the chamber, and drawing it into the cell by means of bibulous paper, placed at its exit. It was found impossible to gauge the volume of solution so introduced,

except in the case of dry blood, and then but approximately. This method of irrigation was adopted, because of the readiness with which alterations, induced by the poisons in living and dead blood, could be followed, step by step, under the microscope, as the irrigation proceeded. Moreover, the contents of the cell, after irrigation, could be kept from contact with air, by sealing the edges of the chamber.

For irrigation purposes, solutions of the sapotoxins in serum, varying in strength from one-half to two per cent, were employed.

MICROSCOPICAL APPEARANCES IN BLOOD TREATED WITH SAPOTOXINS DISSOLVED IN BLOOD SERUM. — When samples of fresh blood, contained in capillary cells, are irrigated with their corresponding sera, holding a sapotoxin in solution, the following changes may be traced, on microscopic examination:

(a.) The erythrocytes become globular, and then fade away somewhat suddenly. The "solution" of the red cells is never quite complete, although their "ghosts" are not easily discernible in unstained preparations.

The "ghosts" of the red cells, after erythrololysis, are clearly brought out, by irrigation with a one-half per cent solution of methylene blue in serum. They are especially conspicuous when dry, but unfixed blood, treated with a serum solution of a sapotoxin, is stained with a serum solution of methylene blue.

The persistence of "ghosts" of red cells in blood laked by the sapotoxins indicates the existence of stroma, a fact which is further corroborated by Stewart's observation, to the effect that in formaldehyde-fixed blood the addition of water does not break up the "ghosts," as is the case when water is added to saponin laked blood.

(b.) The blood-plates disappear, though more slowly than the erythrocytes. Fewer clumps of plates are seen in fresh blood, after treatment with a serum solution of a sapotoxin. With regard to the disappearance of the blood-plates from blood smears, dried at the room temperature, there is

some uncertainty. In spite of the fact that the plates are unfixed, they resist the sapotoxin action to a surprising degree. It is not unusual to find clumps of plates in dry but unfixed blood films, treated with a serum solution of a sapotoxin, after irrigation with methylene blue serum.

(c.) The leucocytes cease at once to exhibit ameboid movements. Those with pseudo-podia retract them, and become more or less globular, and during rapid irrigation roll over and over in the inflowing stream of serum. Many leucocytes are seen grouped together in fields from which the red cells have wholly disappeared.

The granules of the finely granular polymorphonuclear leucocytes become ill-defined and soon fade away altogether. Certain it is that they are no longer stainable. This can be easily verified by irrigating fresh rabbit's blood with rabbit's serum, holding a sapotoxin in solution, until erythrolysis is complete, and then irrigating the laked blood with a decimal five per cent solution of eosin in rabbit's serum. The cytoplasm of the erstwhile finely granular leucocytes now stains diffusely, and shows no vestige of granules. Now, the granules of the majority of granular leucocytes of fresh rabbit's blood, untreated with a sapotoxin, stain readily with a solution of eosin in serum.

But the granules of all the leucocytes are not affected by the sapotoxins to the same extent. Thus in rabbit's blood, the granules of the coarsely granular eosinophilic leucocytes seem to resist the sapotoxin action much longer than the amphophilic granules of the finely granular leucocytes. So that even after prolonged treatment with a sapotoxin, many of these leucocytes show granules that are discrete and stainable.

The sapotoxins exert their solvent action on the granules of unfixed leucocytes only. They are without action on the granules of leucocytes fixed by heat, by formaldehyde, or by alcohol and ether. Even a preliminary irrigation of fresh blood, with a solution of eosin in serum, is sufficient to greatly retard the solvent action of the sapotoxins on the granules of the larger leucocytes. The disappearance of these granules

under the influence of sapotoxins, in serum solution, reminds one of their behavior, when subjected to the action of distilled water. For water alone attacks the granules, although it acts less energetically than serum or water, charged with sapotoxins. When a thin "spread" of rabbit's blood, dried in air, at about 20° C., but unfixed, is treated with pure water, erythrolysis occurs, and the water soon becomes tinged with hemoglobin. If after getting rid of the excess of water, the somewhat altered blood film be fixed in alcohol and ether, and then stained with alcoholic eosin and Nissl's blue, the leucocytes show under the microscope an almost complete absence of granules. The granules also disappear from the leucocytes of fresh blood, subjected to the action of water; but, like solutions of the sapotoxins, water has no solvent action on the granules of heat-fixed or formaldehyde-fixed leucocytes. It is interesting to study the behavior of the leucocytes, when thin films of blood, recently spread and dried at 20° C., are irrigated with solutions in serum of quillaja-sapotoxin or cyclamin. Most of the granular leucocytes lose their granules; and although many of them appear to have diminished in size, their seeming contraction is owing to the fact that their cytoplasm becomes ill defined, making it difficult to trace the contour of individual cells. These are, as a rule, clearly outlined by subsequent irrigation with a solution of methylene blue in serum, and then it becomes apparent that the leucocytes have suffered no appreciable diminution in size. And in cases where the leucocytes had at first remained ill-defined, their contour became distinct after some hours.

In these experiments, care was taken to control all observations, by irrigating dry but unfixed films of blood with a one-half per cent solution of methylene blue in serum, and comparing, under the microscope, sapotoxin-treated leucocytes, stained with methylene blue, with untreated leucocytes similarly stained. Further, unfixed blood films were irrigated with methylene blue serum, and special leucocytes selected for examination. Then, the same films were irrigated with a two per cent sapotoxin solution in serum, and

the effect noted, after which the films were irrigated once more with methylene blue serum. And during this procedure the leucocytes selected were measured.

The nuclei of all varieties of leucocytes, subjected to the action of sapotoxins dissolved in serum, become more distinct and prominent. This, in the case of the granular leucocytes, is mainly due to the disappearance of the granules from their cytoplasm; for in no case could swelling of the nuclei be detected. These retain their staining properties unaltered; and when sapotoxin-laked blood is irrigated with a one-half per cent methylene blue in serum, the nuclei of the leucocytes stain readily, and the arrangement of their chromatin is clearly revealed.

The lymphocytes and large mononuclear leucocytes do not appear to be affected by serum solutions of the sapotoxins.

From the foregoing the conclusion follows that the glucosides under consideration have, of themselves, no leucolytic action; for beyond their solvent action on the granules of the granular leucocytes, no lessening in the number of leucocytes occurs, when unhardened blood is subjected to their action. Indeed, owing largely to the disappearance of the red cells, the leucocytes are especially conspicuous. The persistence of the leucocytes in sapotoxin-laked blood is noteworthy, in view of the fact that blood, so treated, is capable of clotting. It is generally held that many leucocytes break down prior to coagulation, and that fibrin ferment, an essential factor in the process of clotting, is a product of their disintegration. Nevertheless, sapotoxin-laked blood, although extremely rich in surviving leucocytes, is capable of clotting. Many observations were made to establish this point. Samples of blood from various sources, contained in capillary cells, were examined, under the microscope, before and during irrigation with quillaja-sapotoxin dissolved in serum, and any evidence of the disintegration of the white corpuscles carefully sought for, prior to the appearance in the laked blood of fibrin threads. In no case were the leucocytes seen to break down and wholly disappear, showing that coagulation may occur, without being preceded by complete disintegration of indi-

vidual leucocytes. In view of this, it is permissible to suggest that the granules of polymorphonuclear leucocytes may be a source of fibrin-ferment, for the solution of these granules is the only striking change presented by the leucocytes in sapotoxin-laked blood, prior to the onset of coagulation.

It is beyond my purpose to discuss here the function and origin of the blood plates. Suffice it to say that they are regarded by some as a probable source of fibrin-ferment, and it is noteworthy that they rapidly diminish in number, in sapotoxin-laked blood, before the formation of fibrin.

THE ACTION OF THE SAPOTOXINS ON HARDENED BLOOD.

— Many experiments were made with a view to studying the behavior of the formed elements of hardened blood to the sapotoxins dissolved in serum. To this end, blood spreads were fixed, some by heat at 120° C., others by formaldehyde, others again by immersion in a mixture of alcohol and ether, in equal parts. Then, each smear was covered over with a cover glass, in the manner already described, so as to leave a capillary air space above the film of blood. The film was next irrigated, for an hour, with a two per cent sapotoxin solution in serum, during which the formed elements of the blood were watched, at frequent intervals, under the microscope. After treatment with the sapotoxin selected, each film was irrigated with a one-half per cent solution of eosin in blood serum, and, subsequently, with a one-half per cent solution of methylene blue in serum. All three sapotoxins experimented with in this connection gave the same result, of which the following is a summary:

(a.) The red cells preserve their shape, and stain readily when irrigated with eosin-serum. The formaldehyde-fixed red cells, in particular, stain well with eosin, after irrigation with serum solutions of the sapotoxins. As a result of the spectroscopic examination of serum solutions of the sapotoxins, before and after contact with large smears of hardened blood, I am led to the conclusion that the corpuscles, after fixation, either yield no hemoglobin to the sapotoxin charged serum, or that the amount of pigment washed out of

them is insignificant. Again, blood films, fixed in ether and alcohol, then extracted with absolute ether, and then treated with a serum solution of a sapotoxin, show erythrocytes that are perfectly preserved, and that have retained their affinity for eosin. Microscopically, they do not differ from heat-fixed erythrocytes similarly treated. Indeed, from microscopic examination alone, it is impossible to say whether hardened red cells are ever permeable to the sapotoxins; yet from the observations of Stewart, it would appear that formaldehyde-hardened red cells are rendered more permeable by quillaja-sapotoxin, whereas heat-fixed corpuscles remain impermeable.

(b.) All varieties of leucocytes, as well as the blood-plates, resist the sapotoxin action and remain intact. They differ as to none of their histological features from similar elements in hardened blood, which has not been subjected to the action of the sapotoxins. The granules of the granular leucocytes stain perfectly, and the nuclei of all leucocytes remain unchanged as to their staining properties, their structure, and dimensions.

The resistance offered by the erythrocytes of different species to the action of the sapotoxins varies somewhat, a fact which must be borne in mind, when making comparative tests with those glucosides on samples of blood from various sources. The erythrocytes of rabbit's blood are especially prone to yield to the action of hemolytic agents; and the sapotoxins, even when dissolved in serum, induce erythrolysis much more readily in rabbit's blood than in the blood of other laboratory mammals. The red cells of cat's or dog's blood, for instance, are more resistant than those of rabbit's blood. Further, their resistance varies in the same animal according to the degree of alkalinity of its blood. Thus, I find that the blood of meat-fed rabbits is more readily laked than that of the same animals, when kept on a vegetable diet. Rabbits will partake readily of boiled beef, when fed on nothing else. On this diet, their urine, previously turbid and alkaline, becomes clear and acid, usually by the third day. If the alkalinity of the blood be now determined, it

will be found much reduced. The blood of vegetable-fed rabbits is rich in alkaline salts, especially sodium carbonate and alkaline sodium phosphate. On a meat diet there is a diminution in the amount of the latter salt, and a proportional increase in the percentage of acid sodium phosphate in the plasma. Now, acid sodium phosphate, as will appear later, favors hemolysis by the sapotoxins.

RELATION OF THE LAKING ACTION OF QUILLAJA-SAPOTOXIN IN SERUM SOLUTION TO ITS ACTION IN AQUEOUS SOLUTION. — When freshly drawn blood is treated with a solution in serum of one of the sapotoxins, there occurs, within a variable time, complete laking of the blood, provided there be enough of the glucoside in the volume of solution employed to effect complete erythrolysis. But a solution of a sapotoxin in serum acts less energetically, in this respect, than an aqueous solution of the same strength.

The following results show the difference in time taken by quillaja-sapotoxin to completely lake a definite volume of blood, according as the glucoside is dissolved in serum or in water.

TABLE I.

GIVING THE RESULTS OF THE ACTION OF QUILLAJA-SAPOTOXIN, DISSOLVED IN SERUM, ON HUMAN BLOOD. — STRENGTH OF THE SOLUTION .5 PER CENT.

Series A.	Vol. of sol.	Vol. of blood.	Total vol. of serum added.	Weight of sapotoxin in sol.	Time of complete laking.	Coagulation.
A ¹	.1 c.c.	.05 c.c.	.1 c.c.	.0005 gme.	Within 35 seconds.	+
A ²	.075 c.c.	.05 c.c.	.075 c.c.	.000375 gme.	Within 1 minute.	+
A ³	.05 c.c.	.05 c.c.	.05 c.c.	.00025 gme.	Within 1 minute and 25 seconds.	+
A ⁴	.025 c.c.	.05 c.c.	.025 c.c.	.000125 gme.	Within 2 minutes.	+
A ⁵	.025 c.c.	.05 c.c.	.075 c.c.	.000125 gme.	Within 2 minutes.	+

TABLE II.

GIVING THE RESULTS OF THE ACTION OF QUILLAJA-SAPOTOXIN, IN AQUEOUS SOLUTION, ON HUMAN BLOOD. — STRENGTH OF THE SAPOTOXIN SOLUTION 1 TO .2 PER CENT.

Series B.	Vol. of sol.	Vol. of blood.	Vol. of water added.	Weight of sapotoxin.	Time of complete laking.	Coagulation.
B ¹	.01 c.c. of 1 per cent sol.	.05 c.c.	.01 c.c.	.0001 gme.	Within 30 seconds.	+
B ²	.01 c.c. of .2 per cent sol.	.05 c.c.	.01 c.c.	.00002 gme.	Within 1 minute.	+
B ³	.01 c.c. of .5 per cent sol.	.05 c.c.	.01 c.c.	.00005 gme.	Within 1 minute.	+
B ⁴	.05 c.c. of .2 per cent sol.	.05 c.c.	.05 c.c.	.0001 gme.	Within 30 seconds.	—
B ⁵	.05 c.c. of .5 per cent sol.	.05 c.c.	.05 c.c.	.00025 gme.	Within 30 seconds.	—
B ⁶	.05 c.c. of 1 per cent sol.	.05 c.c.	.05 c.c.	.0005 gme.	Within 30 seconds.	—
B ⁷	.1 c.c. of .5 per cent sol.	.05 c.c.	.1 c.c.	.0005 gme.	Within 10 seconds.	—

The foregoing results show that quillaja-sapotoxin, in serum solution, acts more slowly on the red cells, than when dissolved in water. They also emphasize the fact that a small amount of a sapotoxin, dissolved in serum or in a minimum of water, does not materially interfere with coagulation. The laked blood sets into a firm, transparent, clot, which shows strands of fibrin and groups of leucocytes. "Ghosts" of red cells are also seen, but no globular red cells. On the other hand, the addition to blood of an excess of an aqueous sapotoxin solution effectually prevents clotting.

The more rapid laking action of an aqueous solution of a sapotoxin may be accounted for, on the ground that the glucoside, by increasing the permeability of the unhardened red cells, favors water-laking, which occurs more readily in blood diluted with water, than in blood diluted with a corresponding serum. The fact that the red cells become globu-

lar before dissolving, when treated with a serum solution of a sapotoxin, justifies the view that sapotoxin erythrolysis is largely brought about by the passage into the corpuscles of the dissolved substances of the serum, together with its water.

It is possible, however, that water laking is not the sole factor concerned in sapotoxin-laking, for Stewart has shown that sapotoxin laking is producible in sediments of corpuscles, freed as far as possible from serum, and containing little inter-corpuscular water. On this, and on the fact that laking by sapotoxins occurs in blood containing an excess of cane sugar, he bases his conclusion that water-laking is not the primary action of the members of the saponin group. It is quite true that the sapotoxins cause the red cells to become globular and fade away, when blood, containing cane sugar syrup or an excess of sodium chloride, is subjected to their action. The deformed and crenated corpuscles always become globular prior to solution, notwithstanding the fact of their being in hypertonic serum. And the question arises as to whether water-laking can take place in serum or plasma made hypertonic by the addition of sugar or of various salts. Normally, the isotonicity of blood plasma prevents the entrance into the red cells of dissolved substances and water, in quantities sufficient to discharge their hemoglobin. Hence plasma or (after clotting) serum has no softening action on the stromata of the red cells. But any substance, capable of softening their stromata and of lessening the osmotic pressure within them, renders them easily permeable to the water of the plasma or serum. The sapotoxins increase the permeability of the red cells, and favor the occurrence of water-laking, when added to blood, the plasma or serum of which is isotonic or hypotonic. But in a hypertonic serum the tendency is for water to leave, not to enter, the corpuscles, and however permeable these may have become, it is hard to conceive why a more rapid loss of water on their part should be attended by changes characteristic of water-laking. Hence the difficulty in accounting for the primary cause of sapotoxin-laking in hypertonic serum. A possible explanation is the

theory that the sapotoxins combine with the hemoglobin of the red cells to form a compound, readily soluble in the plasma or serum, a compound formed irrespective of the tonicity of the serum or plasma. But there is no evidence of the formation of such a compound. The spectrum of blood, laked by a sapotoxin, is the absorption spectrum of oxyhemoglobin, and its bands occupy identical positions with those of oxyhemoglobin. Further, perfect hemoglobin crystals may be obtained from dog's blood, laked by quillaja-sapotoxin or cyclamin added in substance. So that, if the sapotoxins combine with hemoglobin the resulting compound must be very unstable. But the sapotoxins, without combining with hemoglobin, may act on some constituent of the red cells, of a proteid nature, in the same manner as they act on the granules of the granular leucocytes. Those, it will be remembered, are dissolved by the sapotoxins. This mode of action of the sapotoxins would explain the fact that they exert no softening action on heat-fixed red cells, for they are also without action on the granules of the heat-fixed leucocytes; while the greater rapidity with which the red cells undergo laking in isotonic and hypotonic sera containing a sapotoxin, and the comparative slowness with which they dissolve in hypertonic serum, holding the same amount of that sapotoxin in solution, would seem to indicate that water-laking is ancillary to the specific action of the sapotoxins on a constituent of the erythrocytes.

Some light is thrown on the part played by water-laking during sapotoxin erythrolysis, by studying the behavior of striated muscle-fibers, when these are subjected to the action of a sapotoxin, and noting any alterations in their structure.

Interesting comparisons are afforded by examining, under the microscope, fibers treated with serum solutions of quillaja-sapotoxin and cyclamin, and fibers treated with pure water.

It is known that living muscle quickly loses its excitability under the influence of the sapotoxins. When an excitable frog's sartorius is treated with a sapotoxin in physiological salt solution, contraction, followed by loss of excitability and blanching of the muscular tissue, is observed. When a small

piece of living muscle is teased out in salt solution, and then treated with a two per cent sapotoxin solution in serum, and its effect watched under the microscope, the individual fibers are seen to contract. Then follow structural changes in the fibers themselves. Many fibers present coarse transverse striæ, due to their sarcolemma being thrown into transverse folds. In some fibers, the sarcolemma is partly detached from the sarcous substance so as to form minute blebs. Most of the fibers lose their transverse striæ, but still retain their longitudinal fibrillation. This may be followed by fragmentation of the sarcous substance, especially in the case of heart muscle fibers. The nuclei of the fibers become more prominent, and the protoplasm surrounding them less granular than normal. The fibers also lose much of their pigment simultaneously with the disappearance of their striæ.

Such are the salient changes induced in striped muscle by the sapotoxins in serum solution. They are identical with the structural changes induced in the fibers by pure water. Water causes water rigor and partial detachment of the sarcolemma from the sarcous substance of the fibers. Many fibers lose their transverse striation, and their nuclei stand out more clearly. The fibers also lose much of their hemoglobin. Judging, therefore, from the histological changes alone, the alterations which appear in the fibres, after treatment with a sapotoxin, must be put down to the entrance of water into their substance. The increased permeability of the fibers, after a sapotoxin, is due to a special action on the sarcolemma or on the sarcous substance. In view of what happens when hardened blood films are acted upon by the sapotoxins, it is interesting to note that voluntary muscle fibers, fixed with formaldehyde or other fixing agents, are not in the slightest degree affected by the sapotoxins, the fibers neither losing their pigment nor their striæ.

IS THE CHOLESTERIN OF THE CORPUSCLES ATTACKED BY THE SAPOTOXINS? — The subject of the special action of the sapotoxins on some constituent or constituents of the red

cells has been investigated by Ransom. He believes that members of the saponin group combine with the cholesterin and lecithin of the corpuscles. This mode of action of a sapotoxin is apparently confirmed by Stewart's finding,⁸ that while quillaja-sapotoxin produces no change in the conductivity of a suspension of formaldehyde-fixed red cells, extracted with ether, it increases the permeability and conductivity of the unextracted corpuscles. The conclusion follows that the increase of permeability in the unextracted formaldehyde corpuscles, caused by quillaja-sapotoxin, is due to its action on a substance or substances soluble in ether.

In spite of these results, there is considerable doubt as to whether cholesterin plays any part in the neutralization or fixation of any of the sapotoxins. We are led to this belief by results obtained with solutions of quillaja-sapotoxin and cyclamin, after contact with anhydrous cholesterin (Merck's) and cholesterin mixed with lecithin. The following is a summary of our results:

(a.) An excess of cholesterin was added in substance to a one-half per cent solution of quillaja-sapotoxin in rabbit's serum, and the mixture, after shaking, was allowed to stand twenty-four hours in a cool place. At the end of that time, a heavy sediment of cholesterin was found in the tubes charged with the mixture, scarcely any of it having dissolved in the serum. The clear supernatant serum solution of sapotoxin was now drawn off, and its erythrolytic action tested. In every case marked erythrolysis was observed, the sapotoxin serum acting as though it had not been shaken with pure cholesterin. Moreover, the serum treated with cholesterin was injected intravenously into rabbits, in amounts varying from one-half to one and one-half cubic centimeters, and with results identical with those obtained on injecting sapotoxin-serum, not previously shaken with cholesterin. All the symptoms of sapotoxin intoxication ensued; and within an hour nucleated red cells had appeared in the circulation. Rabbits that had received one and a-half cubic centimeters of the cholesterin-treated serum, containing

sapotoxin, succumbed; and the changes characteristic of acute sapotoxin poisoning, such as subserous extravasations of blood, punctiform hemorrhages, softening and hyperemia of the marrow of the long bones, hyperemia and necrosis in various organs, were found post mortem.

Similar results were obtained with cyclamin in serum after twenty-four and forty-eight hours' contact with an excess of cholesterin.

(*b.*) In order to bring the cholesterin into more intimate contact with the serum solution of quillaja-sapotoxin, the cholesterin was first treated with oleic acid, and the resulting compound added to the serum in excess. The oleic ether of cholesterin is liquid at the room temperature, and when shaken with serum, or with a serum solution of a sapotoxin, gives a perfect emulsion, having the color and odor of lanolin. Such an emulsion is powerfully erythrolytic, and proves fatal to rabbits when injected intravenously, the animals dying not of embolism but of sapotoxin poisoning, as evinced by the blood and tissue changes.

(*c.*) A one per cent solution of quillaja-sapotoxin, in nine-tenths per cent sodium chloride solution, was prepared.

Five cubic centimeters of this solution were shaken with two cubic centimeters of a saturated solution of cholesterin in chloroform, and five cubic centimeters more were shaken with two cubic centimeters of a saturated solution of cholesterin in ether. On agitation, these mixtures gave white emulsions, the emulsion in the former case being much thicker than in the latter. After standing twenty-eight hours in a cool place, the mixture of sapotoxin-serum with cholesterin, dissolved in ether, was found to have separated into two distinct layers, the lower one of which was aqueous and free from opalescence; whereas the mixture, containing cholesterin in chloroform, showed a slightly opalescent fluid on the surface of a thick emulsion. The aqueous part of each mixture, after separating off the ether or chloroform emulsion of cholesterin was heated on the water-bath to drive off any trace of ether or chloroform and after cooling its erythrolytic action was tested. By way of control, the

erythrolytic action of a one per cent sapotoxin in salt solution, shaken with pure ether, and that of a one per cent solution, shaken with pure chloroform, were also tested. An emulsion was obtained with chloroform alone, but not with pure ether.

The clear fluid, from the mixture of sapotoxin solution with cholesterin in ether, scarcely frothed on shaking, and was found to have largely lost its hemolytic action. One must not, however, infer from this that cholesterin exercises a neutralizing action on quillaja-sapotoxin; for ether alone extracts the sapotoxins from their aqueous solutions, as proved by the fact that the residue from the ethereal extract, after evaporation to dryness, gives with sulphuric acid and bromine water the characteristic reaction of the sapotoxins. Moreover, the clear fluid from the mixture of sapotoxin solution and ether shows marked impairment of hemolytic potency.

Chloroform also removes the sapotoxins from their solution in water.

These results lead one to infer that the seeming neutralization of quillaja-sapotoxin by cholesterin, when a solution of the glucoside is shaken with a solution of cholesterin in ether or chloroform, is due to the extraction of the sapotoxin by the ether or chloroform, and not to any neutralizing action of cholesterin.

Stewart lays stress on the fact that extraction with ether of formaldehyde-fixed corpuscles prevents sapotoxin from increasing the permeability of the corpuscles, and from this he infers that quillaja-sapotoxin increases the permeability of the unextracted formaldehyde corpuscles, by an action on substances soluble in ether. Now, when a sediment of formaldehyde fixed corpuscles is extracted with ether, the cholesterin and lecithin removed is largely derived from the leucocytes, and after ether extraction there is a dearth of leucocytes. They are an important source of cholesterin and lecithin in the blood, and failure of a sapotoxin to increase the permeability of formaldehyde fixed red cells, after extraction with ether, is due to the thorough hardening of

the corpuscles by ether, and not to the removal of cholesterolin from their substance. "Microscopically, the corpuscles are well preserved, very distinct, smooth, and round in outline, and they retain all their blood pigment. On the average the corpuscles are somewhat smaller than in the original washed formaldehyde sediment. They preserve the normal dumb-bell shape when seen on edge. They are tinged more deeply with blood pigment than the unextracted corpuscles." (Stewart.) The fact is, that the combined action of formalin and ether is to harden the red cells as thoroughly as they are hardened when fixed by heat, and the sapotoxins are admittedly without action on heat fixed corpuscles.

There is no proof, to my mind, that cholesterolin is capable of neutralizing the sapotoxins and rendering them inert. Nor is lecithin capable of neutralizing them; for crude cholesterolin, rich in lecithin, is as inactive, in this respect, as pure cholesterolin.

Although cholesterolin plays no part in counteracting the laking action of sapotoxins, certain salts can inhibit or accelerate their action, according as they diminish or increase the permeability of the red cells to substances dissolved in the serum. A study of the behavior of the red cells toward acid sodium phosphate, sodium chloride, and ammonium chloride led me to inquire as to whether the hemolytic activity of quillaja-sapotoxin and cyclamin was in any way influenced by them.

Known quantities of acid sodium phosphate and sodium chloride were added to solutions in serum of sapotoxins, and the time required for complete laking of a measured volume of blood, by a known volume of a salted sapotoxin solution, was noted. The details are set forth in tables III., IV., V., and VI.

TABLE III.

GIVING THE RESULTS OF THE ACTION OF QUILLAJA-SAPOTOXIN, DISSOLVED IN SERUM, ON HUMAN BLOOD, BEFORE AND AFTER THE ADDITION OF ACID SODIUM PHOSPHATE. STRENGTH OF SOLUTION .18 PER CENT.

Series C.	Vol. of sol.	Vol. of blood.	Weight of sapotoxin.	Percentage of H_2NaPO_4 .	Time of complete taking.	Coagulation.
C ¹	.05 c.c.	.05 c.c.	.00009 gme.	—	Within 6 minutes.	+
C ²	.05 c.c.	.05 c.c.	.00009 gme.	1 per cent	Within 5 minutes.	—
C ³	.05 c.c.	.05 c.c.	.00009 gme.	2 per cent	Within 4 minutes.	—
C ⁴	.05 c.c.	.05 c.c.	.00009 gme.	4 per cent	Within 3 minutes.	—
C ⁵	.05 c.c.	.05 c.c.	.00009 gme.	5 per cent	Within 3 minutes.	—
C ⁶	.1 c.c.	.05 c.c.	.00018 gme.	1 per cent	Within 3 minutes.	—
C ⁷	.1 c.c.	.05 c.c.	.00018 gme.	2 per cent	Within 2 minutes.	—
C ⁸	.1 c.c.	.05 c.c.	.00018 gme.	3 per cent	Within 1 minute and 30 seconds.	—
C ⁹	.1 c.c.	.05 c.c.	.00018 gme.	4 per cent	Within 1 minute and 30 seconds.	—
C ¹⁰	.1 c.c.	.05 c.c.	.00018 gme.	5 per cent	Within 1 minute and 30 seconds.	—

TABLE IV.

GIVING THE RESULTS OF THE ACTION OF QUILLAJA-SAPOTOXIN, DISSOLVED IN SERUM, ON HUMAN BLOOD, BEFORE AND AFTER THE ADDITION OF SODIUM CHLORIDE. STRENGTH OF THE SOLUTION .18 PER CENT.

Series D.	Vol. of sol.	Vol. of blood.	Weight of sapotoxin.	Percentage of NaCl.	Time of complete laking.	Coagulation.
D ¹	.05 c.c.	.05 c.c.	.00009 gme.	Control test. No salt.	Within 6 minutes.	+
D ²	.1 c.c.	.05 c.c.	.00018 gme.	Control test. No salt.	Within 2 minutes and 30 seconds.	+
D ³	.1 c.c.	.05 c.c.	.00018 gme.	1 per cent	Within 6 minutes.	—
D ⁴	.1 c.c.	.05 c.c.	.00018 gme.	2 per cent	Within 9 minutes.	—
D ⁵	.1 c.c.	.05 c.c.	.00018 gme.	3 per cent	Within 11 minutes.	—
D ⁶	.1 c.c.	.05 c.c.	.00018 gme.	4 per cent	Laking not complete after 12 minutes.	—
D ⁷	.1 c.c.	.05 c.c.	.00018 gme.	6 per cent	Laking not complete within 15 minutes.	—

TABLE V.

GIVING THE RESULTS OF THE ACTION OF CYCLAMIN, DISSOLVED IN BLOOD-SERUM, ON HUMAN BLOOD, BEFORE AND AFTER THE ADDITION OF ACID SODIUM PHOSPHATE. STRENGTH OF THE SOLUTION .18 PER CENT.

Series E.	Vol. of sol.	Vol. of blood.	Weight of cyclamin.	Percentage of H_2NaPO_4 .	Time of complete laking.	Coagulation.
E ¹	.05 c.c.	.05 c.c.	.00009 gme.	Control test. No salt.	Within 2 minutes.	+
E ²	.05 c.c.	.05 c.c.	.00009 gme.	.5 per cent.	Within 1 minute.	—
E ³	.05 c.c.	.05 c.c.	.00009 gme.	1 per cent.	Within 35 seconds.	—
E ⁴	.05 c.c.	.05 c.c.	.00009 gme.	2 per cent.	Within 35 seconds.	—
E ⁵	.05 c.c.	.05 c.c.	.00009 gme.	4 per cent.	Within 30 seconds.	—
E ⁶	.05 c.c.	.05 c.c.	.00009 gme.	6 per cent.	Within 30 seconds.	—

TABLE VI.

GIVING THE RESULTS OF THE ACTION OF CYCLAMIN, DISSOLVED IN SERUM, ON HUMAN BLOOD, BEFORE AND AFTER THE ADDITION OF SODIUM CHLORIDE. STRENGTH OF THE SOLUTION .0375 PER CENT.

Series F.	Vol. of sol.	Vol. of blood.	Percentage of NaCl.	Weight of cyclamin.	Time of complete laking.	Coagulation.
F ¹	.05 c.c.	.05 c.c.	Control test. No salt.	.00001875 gme.	Within 6 minutes.	+
F ²	.1 c.c.	.05 c.c.	Control test. No salt.	.0000375 gme.	Within 1 minute and 30 seconds.	+
F ³	.05 c.c.	.05 c.c.	3 per cent.	.00001875 gme.	Within 15 minutes.	—
F ⁴	.1 c.c.	.05 c.c.	3 per cent.	.0000375 gme.	Within 2 minutes.	—
F ⁵	.1 c.c.	.05 c.c.	6 per cent.	.0000375 gme.	Within 2 minutes.	—
F ⁶	.1 c.c.	.05 c.c.	12 per cent.	.0000375 gme.	Within 5 minutes.	—

From the findings just recorded the following inferences may be drawn:

(1.) Sodium chloride delays the hemolytic action of quillaja-sapotoxin and cyclamin, and acid sodium phosphate increases that action.

(2.) The influence of either salt is proportional to its concentration.

SUMMARY.

(1.) Quillaja-sapotoxin, cyclamin, and saporubrin are powerfully erythrolytic.

(2.) Their potency depends on several factors:

(a.) On their purity.

(b.) On the nature of the media in which they are dissolved; they are more active in hypotonic than in isotonic solutions; they are more active in aqueous solutions than in serum.

(c.) On the presence of salts capable of affecting the permeability of the red cells.

(3.) They liberate hemoglobin from the red cells whether these be moist or dry; but they are without action on the stromata of red cells, fixed by formaldehyde after drying at the room temperature, and of the red cells fixed by heat, or by alcohol and ether.

(4.) Acting as protoplasmic poisons, they quickly kill the leucocytes, causing cessation of their movements. They also exert a solvent action on the granules of granular leucocytes, but do not impair the staining properties of their nuclei.

(5.) They do not inhibit the disintegration of the blood-plates in fresh blood; but they are without action on the plates after fixation.

(6.) Dissolved in blood serum they slightly delay the onset of clotting.

(7.) Cholesterin plays no part in the neutralization of the sapotoxins studied.

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SIMPLE ADENOMA OF THE PANCREAS ARISING FROM AN
ISLAND OF LANGERHANS.

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Royal Victoria Hospital, Montreal.)*

The tumor that I have the opportunity of describing in the present communication was discovered accidentally by me at autopsy, and I have been moved to place it on record in the belief that it is unique, a somewhat careful study of the literature on the subject failing to reveal any case even approximating to the condition.

One is struck in looking over the overwhelming mass of publications on pancreatic disease with how little attention has been paid to the progressive disturbances of the gland and notably tumor formation. Even yet we have but little information as to the place and mode of origin of carcinomata, the most frequent neoplasm of the pancreas, and still less is known with regard to the simple or benign growths. This is chiefly, I believe, due to the fact that at autopsy the pancreas generally receives but scant attention, so that many conditions that might throw light upon the etiology of gross disease are overlooked. This is the more unfortunate since it is frequently the study of lesions in the earlier stages that gives us the most valuable information. Primary tumors of the pancreas are certainly not common. Thus Remo Segie¹ in eleven thousand five hundred autopsies at Milan found only one hundred and thirty-two instances, a proportion of one and fourteen-hundredths per cent. As may be seen by a reference to any standard text-book on pathology, carcinoma is practically the only tumor that receives consideration, owing of course to its relative frequency. With regard to the ratio of occurrence of the various neoplasms, the only figures I have to refer to are those of Remo Segie just mentioned. In his one hundred and thirty-two cases he found cancer one hundred and twenty-seven times; sarcoma twice; cysts twice;

syphiloma once. Combining the statistics of the Royal Victoria and the Montreal General Hospitals, in fifteen hundred and fourteen autopsies of which I have notes, primary carcinoma occurred six times and adenoma once. With regard to carcinoma alone, Förster in six hundred and thirty-nine post-mortems found eleven cases, a proportion of nine-tenths of one per cent. The only references to benign growths I have been able to find in any continued article on the subject are in Oser's monograph on the Pancreas in Nothnagel's "*Specielle Pathologie*," where three cases are cited to which I shall refer shortly.

The tumor that I here desire to record was a small, round, and somewhat flattened nodule on the anterior surface of the pancreas, situated about the junction of the middle and terminal thirds. It first attracted attention by its color, which was tawny yellow with a few distended blood-vessels upon the surface, contrasting sharply with the ivory white of the pancreas proper. When cut into, it was well-circumscribed, rather soft, and exuded a little blood. The whole mass was not larger than a marrowfat pea.

MICROSCOPICAL EXAMINATION.—The portion of the pancreas containing the tumor was hardened in five per cent formalin and mounted in paraffin. In spite of great care taken in embedding, the cells of the tumor became somewhat shrunken, but it was nevertheless possible to get a good idea of the original structure of the growth.

The sections were cut on a Minot microtome and stained with hematoxylin, hematoxylin-eosin, thionin-blue, and Mallory's stain for connective-tissue. When mounted the tumor presented as a perfectly oval nodule in the pancreatic substance, measuring three by two and one-half millimeters, and projecting somewhat above the general level.

The tumor was enclosed completely in a delicate capsule composed of wavy laminæ of connective-tissue rather loosely arranged and with relatively few nuclei. In this capsule were narrow elongated cavities lined with flattened cells containing spindle-shaped nuclei. Some of these cavities contained

blood and were clearly blood-sinuses, while others appeared to be lymph-spaces. In addition, in the capsule could be seen small groups of cells resembling those of the pancreatic acini, but flattened, compressed, and atrophic. At one spot outside the main mass was a small nodule enclosed in a separate thin capsule and identical in structure and appearance with the tumor proper. This was possibly due to the existence of a minute subsidiary growth beside the main one or to a slight obliquity in the cutting of the section. It was certainly not an infiltration in the sense of malignity, for the growth was quite regular, the capsule was perfect, and not involved in the extension of the new-growth.

Viewed as a whole with the low power (No. 3 Winkel), the tumor was composed of a great number of cell-masses bounded by more or less completely anastomosing bands of fibrous tissue so that a somewhat alveolar-looking stroma was produced. These fibrous bands did not always join, but some formed isolated irregularly-branching and stellate masses. In the center were what at first sight appeared to be sinuses filled with blood. The cells filling the alveolar spaces had a general resemblance to the cells of the pancreas. They formed rounded, elongated, and irregular clumps, and in some cases a single row which branched freely or followed a sinuous course in close touch with the ramifications of the stroma. In a few places a tendency to form lumina was observed, but this was only apparent, as in the majority of cases a single small mass of connective-tissue could be made out in the center. The cells in question were not in close contact with the supporting stroma, but had shrunk, no doubt in the process of embedding. The general appearance of the growth, which recalls somewhat the tubular adenomata of the kidney, may be gathered very well from the figure (Plate XVIII., Fig. 1).

With the high power (Winkel No. 7), the supporting stroma was found to be composed of a slightly cellular connective-tissue forming irregular masses from which delicate prolongations passed out to meet those of similar masses near by. The nuclei here were round or bluntly spindle,

and the bands contained minute blood-vessels. The "blood-sinuses" mentioned proved not to be true sinuses, inasmuch as there was neither limiting membrane nor endothelium, but were simply areas of blood-extravasation between the cells, which were thereby dislocated, and a false impression of lumina was thus produced. The cells adjoining these extravasations were distinctly compressed. A considerable amount of amorphous blood pigment was here observed.

Coming to the cells proper of the growth, they varied somewhat in shape, some being short columnar, others being polyhedral, depending on the arrangement whether in columns or in masses. The cytoplasm was granular, taking the eosin fairly well. The nuclei were pale, somewhat vesicular, presenting several dots of chromatin much darker than the rest. In some there was a central darker nucleolus from which radiating bands of chromatin passed to the periphery. The peripheral portion was also darker than the rest. The nuclei were for the most part rounded, or irregularly oval, and rather large. As compared with the cells of the pancreatic acini, the cells were smaller, the nuclei relatively larger, and the cytoplasm much looser in texture, staining both more faintly and more irregularly. The strands of the supporting stroma were much coarser in the tumor than those between the acini. In the preparation stained by thionin, the peculiarities mentioned came out with even greater distinctness (Plate XVIII., Fig. 2). The cytoplasm of the epithelial cells of the tumor was faintly tinged with blue and showed numerous dots staining intensely. The reticular arrangement of the chromatin was well brought out.

We have, therefore, briefly, to do with a tumor consisting of a stroma of connective-tissue arranged in the form of imperfect and irregular alveoli, in the interstices of which are situated cells of a glandular type, forming masses and wavy bands. From this structure it is clear that the tumor is adenomatous in character. Again, from the fact that the growth is well encapsulated and shows no tendency to infiltrate or take on aberrant growth, it is clearly benign. Nowhere is the capsule invaded. The term **SIMPLE ADENOMA** would therefore correctly describe it.

For purposes of comparison I would refer just here to the few cases of adenoma of the pancreas hitherto recorded.

Owing to the kind coöperation of Prof. J. G. Adami, I have been enabled to abstract, I believe, the complete literature on the subject, but find that it throws but little light on it. So far as I am aware, nothing like the above growth has been described.

Thierfelder^I found in the otherwise normal head of the pancreas of a young man who died of tuberculosis a firm, relatively bloodless tumor the size of a cherry, which he was able to remove intact from its fibrous capsule. The tumor was composed of much winding and dividing cell-cylinders possessing no lumina. The stroma was in the main firm and poor in cells. Here and there, however, it was looser and more cellular. In the centre of the mass was a small calcified area. According to Thierfelder's description the cells of the new-formation resembled the cubical epithelium of the smaller glands and excretory ducts, and were from their arrangement to be regarded as originating from these. The figure Thierfelder gives is so poor that very little can be made of it. The appearances indicated, however, are those of a somewhat scirrhus carcinoma rather than a simple growth.

Neve^{IV} records under the head of "adenoma" the following case: In a female, aged fifty, a globular tumor was found in the region of the pancreas. This measured two by two and one-half inches, and was somewhat flattened at the poles. The growth was adherent to the duodenum which was narrowed. The bile-duct was also included and contracted. To quote Neve's own description there were "microscopically trabeculæ of nucleated fibrous tissue. Spaces lined by well nucleated cells. In some places fibrous tissue presented scirrhus characters. Here and there were atrophied lobules of glandular tissue. In the center of some of the alveolar spaces there are larger cells, some measuring .025 m.m. These appeared later to lose their nuclei and become granular and yellowish masses, retaining their original shape. In some places glandular tubes (apparently new-formed) were to be seen packed with round cells. Others were older and

full of structureless material." This description does not make it absolutely certain that the tumor in question was an adenoma. It might equally well have been carcinomatous, so that it must be included, like Thierfelder's, with the doubtful cases.

The case reported by A. Cesaris-Demel^V may, however, be accepted with considerable certainty as being a true adenoma. His patient was a male, aged sixty, who died of purulent cystitis and pyelonephritis. He presented well-marked evidences of syphilis, such as abundant osteophytes on the inner table of the skull, general atheroma, scars on the liver, and fibrosis of the spleen and pancreas.

The pancreas was notably attenuated and almost completely substituted by compact adipose. The glandular substance was markedly diminished and replaced by hard, compact tissue of connective-tissue type, in which the acinous structure could be distinguished. In the inferior convex border of the organ about the middle was a tumor the size of a pigeon's egg which was free from adhesions. The surface was nodular. The mass was enclosed in a firm fibrous capsule about one millimeter thick. The tumor proper was soft and fleshy, very vascular, the center homogeneous and hyaline, from which passed radiating septa. The lymph-glands in the neighborhood were scarcely recognizable and there were no metastases. Microscopically, the cells of the tumor were for the most part cylindrical, but in some regions irregular as if from pressure, with an imperfect reproduction of an excretory tubule. There were no cell-inclusions and no nuclear degeneration. The author remarks that he could find no other cases in the literature and has no doubt that he had to do with an acinous adenoma. On a reference to his figures it would appear that his view is correct. The structure suggests a growth starting from the excretory ducts.

Biondi^{VI} records a case that he calls a fibro-adenoma. His patient, aged forty-five, had been in good health until six years previously, when she began to suffer with anorexia, nausea, eructations, continuous but not severe epigastric pains, progressive emaciation, and the presence of a tumor.

She was sub-icteric, and a tumor the size of a hen's egg was discovered in the middle of the epigastrium. There were neither hypo- nor hyper-chlorhydria. From the pain and emaciation, tumor of the pancreas was diagnosed. Bronzing of the skin also suggested pressure on the solar plexus.

The tumor was found in the head of the pancreas and was well encapsulated and delimited. The surface had a curious mother-of-pearl appearance. The tumor was removed and the patient remained in excellent health for three years and nine months. The growth was in the main fibrous, but contained spaces or canals having peripheral digitations, lined by a single layer of cylindrical epithelium. Within the canals was a granular material. The more central softer part showed tortuous cylindrical convolutions also lined with a single layer of epithelium, the characteristics of which were those of the pancreatic ducts.

A few other cases may be mentioned, but are with great hesitation to be included in the category of adenomata. Such is that of Baudach,^{III.} who describes a hemorrhagic cyst of the pancreas that developed within an adenomatous new-growth with marked overgrowth of the vessels and secondary myxomatous degeneration.

Martin² reports a case that was possibly adenomatous.

Another is that of Ruggi,^{II.} where in a woman fifty years of age two tumors were found attached by pedicles to the pancreas and removed. One of these weighed six hundred and fifty grams. The diagnosis was "adenosarcoma." From the appearance of the figures given, the tumor is almost certainly not a plain adenoma, but rather an adenocarcinoma.

Cases such as those of Thierfelder, Neve, and Ruggi illustrate the difficulty of drawing a hard and fast line between benign and malignant growths in certain cases. There appear to be certain nodular growths of the pancreas that histologically resemble carcinoma very closely, if indeed they be not identical with it, and yet these are well encapsulated and exhibit no tendency to infiltrate or form metastases. Possibly many of these go on to form true cancer, but so little is known of this subject that it is as yet a matter

of conjecture whether carcinoma of the pancreas takes its origin in a previously existing adenomatous growth or whether it may start immediately from the specific cells of the acini or ducts. In such cases when the tumor is removed during life, probably the only clue we can get to the true state of affairs is in the formation of metastases.

In my own example a point of great interest and importance was to determine its origin.

Glandular tumors of the pancreas, such as those described, must of necessity arise from one of three structures: (1) the epithelium of the ducts, (2) the pancreatic acini, (3) the islands of Langerhans. There is one possible exception that will be referred to shortly.

The adenomata of the pancreas hitherto described appear to have been derived from the ducts, the epithelial elements being composed usually of short columnar cells and having a tendency to form a lumen, as most growths of this type do. In the tumor that I have here described, however, the gland-cells, while in a few instances they are short and columnar, bear no resemblance to those of the ductal epithelium, since they are granular, the cytoplasm staining more deeply, and form no true lumina. They also differ from the lining cells of the finer excretory ducts in which the cytoplasm is relatively small as compared with the nucleus. It only remains to decide whether it originated in the acini or in an island of Langerhans, and I think it possible to determine this with certainty.

In looking over microscopic preparations of the pancreas, no one familiar with the appearance of the organ can have failed to notice the differences both in structure and arrangement of the cells of the islands as compared with those of the acini. The differences are apparent in sections prepared by all methods, but are specially well-marked in those stained by Van Gieson, thionin-blue, and Mallory's connective-tissue stain. The islands invariably appear as more or less rounded collections of cells staining much more lightly than the rest of the pancreas, and sometimes bounded by a thin connective-tissue membrane. The cells composing them are poly-

hedral and flattened rather than pyramidal. The cytoplasm does not take the dye so diffusely and appears pale, almost colorless, with deeper staining granules, whereas the cytoplasm of the acinar secreting cells takes nuclear stains rather deeply. The nuclei also do not stain so intensely or so homogeneously as those of the cells of the acini, but are more vesicular-looking, and contain well-marked nucleoli and numerous dots and threads of chromatin. A further point of distinction, well brought out by Mallory's method, is that while the connective-tissue of the lobules where it surrounds the acini is delicate and thread-like, in the islands much thicker bands can be made out, containing capillaries and showing a tendency to branch. Another difference is that the cells of the island do not form groups about a central minute lumen, but form irregular masses and wavy anastomosing columns between which the connective-tissue stroma appears. In those preparations stained by Mallory's method one notices that the islands are of a pale dull brown color contrasting with the steel-blue of the acini. Again, the nuclei of the acinar cells stain very diffusely and indistinctly, but those of the cells in the islands are clear, vesicular, and take the orange constituent of the stain particularly well. The cytoplasm is a dirty bluish-brown, a fact which explains the brownish color of the islands under the low power. When we study the tumor in question in the light of these facts we see that it resembles an island of Langerhans far more than an acinus. In fact, so far as the arrangement of the glandular cells, their structure and peculiarities of staining, and the appearance of the fibrous stroma, are concerned, the tumor rather suggests an overgrown island. Stained by Mallory's method the tumor stands out as a clearly defined brownish nodule against the blue of the rest of the pancreas. The stroma is in the form of thick branching trabeculæ containing vessels; the specific cells are clear in appearance, the nuclei taking the orange stain rather deeply, and are of a bluish-brown tinge, so that the whole appearance of the structure is identical morphologically and microchemically with that of the cells of the island of Langerhans. To my mind it is impossible to resist

the conclusion that the tumor in question originated in the overgrowth of an island.

How these pancreatic adenomata start is difficult to explain. The case of Cesaris-Demel is interesting as it suggests a clue to the development of some at least of the growths. Here we note atrophy of the glandular tissue of the pancreas with considerable fibrosis and substitution of the specific tissue with fat that were attributed to syphilis. It may be that, as in the parallel case of atrophic cirrhosis of the liver where we have an imperfect replacement of the destroyed lobules in the shape of warts or adenomata, so here the tumor may have had its origin in a compensatory hyperplasia of certain of the specific cells of the pancreatic acini. Such could not have been the explanation, however, in my case, since the pancreas was in other respects normal. Further I could not make out any degeneration or numerical diminution in the islands of Langerhans. It is possible that some at least of the nodular tumors of the pancreas, notably those of angiomatous structure, are derived not from the pancreatic cells proper but from suprarenal "rests" included at some time within the organ. Questions of etiology, however, cannot be settled until more material has accumulated. Unfortunately these tumors are described but seldom, for the smaller ones may be readily overlooked by the pathologist and the larger growths only come under the ken of the clinician when they are of such a size and in such a position as to press upon important structures.

It is however certain that many of the "cysts" of the pancreas are properly to be included in the group of adenomata. This subject is as yet in great confusion. Surgeons are apt to regard every cyst in the neighborhood of the pancreas as of pancreatic origin, and no doubt many conditions differing widely etiologically are brought into the same category. Here again the study of our morbid material is deficient. Retention cysts, of which the so-called "acne pancreatica" and "ranula pancreatica" are examples, as well as hemorrhagic and other degeneration cysts, are not uncommon, but "proliferation" cysts or cystadenomata are

decidedly rare. Garrigues³ describes a "cystadenoma" of large size which Birch-Hirschfeld was inclined to regard as a true cystadenoma. The tumor was multiloculated, the principal cyst containing two and a half gallons of fluid. The walls of the cavities showed large holes and were lined by cylindrical or polygonal epithelium. Somewhat similar cases are recorded by Riedel,⁴ Salzer and Paltauf,⁵ Nimier,⁶ and Heaton.⁸ Fitz⁷ and Moynihan⁹ have written lengthy papers on the subject. Judging from the published cases there are two varieties at least of pancreatic cystadenomata: multilocular cystomata and papillomatous cystomata (see Ransohoff¹²). As will be observed, the analogy with the ovary is close. Just as in the case of the solid adenomata so here it is difficult to decide just where malignancy begins. The so-called "cystic epitheliomata" of the pancreas, of which cases have been recorded by Hartmann,¹⁰ Terrier (quoted by Nimier, loc. cit.), Gilbert,¹¹ appear invariably to be malignant.

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A STUDY OF EEL SERUM, AND THE PRODUCTION OF AN
ANTITOXIN IN A COLD-BLOODED ANIMAL — A CONTRI-
BUTION TO THE STUDY OF IMMUNITY.

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Investigations in the domain of immunity are increasing upon all sides in search of the laws that govern this intricate problem, and each investigation adds some fact to our knowledge. Since Metchnikoff's classical experiments demonstrating immunity against microorganisms were confirmed, scientists have turned their attention to immunity against microbial toxins and other closely allied substances.

Shortly after A. Mosso¹ had published his observations upon the toxicity of eel serum, this substance was called upon by experimenters to contribute to the study of immunity and has served for several interesting researches upon this subject. According to A. and U. Mosso,² serum of the eel is a transparent liquid of greenish blue color and a strongly alkaline reaction; its toxic power is destroyed at 70° (Cent.), also by mineral acids and the alkalis. A fatal dose injected into the marginal vein of the ear of the rabbit causes that animal to make convulsive movements accompanied with exophthalmia, contraction of the pupil, salivation, hematuria, and epistaxis; finally the heart is arrested in systole and the animal dies. If the dose is feeble the animal falls into a state of cachexia and succumbs after a few days. As it is entirely beyond the scope of this paper to describe the chemistry and physiological action of eel serum, we will content ourselves with the brief notice above given and pass immediately to the experiments we have undertaken.

We consider one-tenth of a cubic centimeter per kilogramme of rabbit as the lethal dose of eel serum, that is, of fresh serum, as its toxicity diminishes; for example, after it has aged one month two-tenths of a cubic centimeter, and

even three-tenths of a cubic centimeter, per kilogramme can be injected into a rabbit without exciting any immediate effect, and sometimes the animal will escape the remote consequences. Camus and Gley³ have insisted upon the color of the serum as indicative of its toxicity, and express themselves by saying: "The serum collected is usually of a beautiful greenish blue color, sometimes yellowish. In this latter instance we have always found it less toxic." We have often observed these different shades, but they have never corresponded to different degrees of toxicity, which has always depended upon the age of the serum. In passing it might be well to call attention to the fact that the eel yields more blood in the spring and summer than in winter, but the toxic value of the serum collected in these seasons is the same.

In extracting blood from the eel we have followed almost exactly the technic of other experimenters, which is to ligate the aorta, and then penetrate that vessel with a finely pointed sterile pipette; to prevent all infection it is necessary to cauterize the artery before making the puncture, for we have invariably remarked the presence of the *staphylococcus pyogenes aureus* in the pericardial fluid.

Once the blood has been collected it is left for twenty-four hours at the laboratory temperature, at the end of which time a layer of clear serum will have collected above the deposit of red globules. It is now time to draw off this serum into another tube, otherwise it will begin to dissolve the red globules. By these means the blood yields just as much and as satisfactory a serum as if it had been immediately centrifugalized. Lastly by diluting one part of serum with nine parts of physiological salt solution, it is ready to serve for injection.

The rabbit is the animal of choice for immunization, for although it is extremely sensitive to the toxic action of eel serum, yet with care and prudence it can be thoroughly immunized. We have made many trials with the guinea-pig, but, like our predecessors, without success. This latter animal has a greater resistance to the immediate effects of

the eel serum than the rabbit; thus one-tenth of a cubic centimeter of eel serum, diluted with one cubic centimeter of physiological salt solution and injected into the marginal vein of a rabbit weighing sixteen hundred and twenty grammes, causes its death in nine minutes; while the same dose injected into the jugular vein of a guinea-pig weighing four hundred and forty-five grammes does not kill the animal under five hours after the injection. But these results are reversed when we consider the remote effects, for after the injection of a minute dose (twenty-five hundredths of a cubic centimeter) of eel serum the guinea-pig progressively loses weight and after several days dies of marasmus. All our attempts to immunize the guinea-pig have been in vain; even with eel serum heated to sixty degrees Centigrade, during five, ten, twenty, and thirty minutes, in minute and gradually augmented doses, injected at short and long intervals, a failure has been the invariable result.

Contrary to Tchistovitch,⁴ who states that the rabbit is very easy to immunize, we have found that in order to succeed with this animal it is necessary to exercise the greatest care. Some experimenters advise the injection of a minute dose at the commencement, and then waiting until the animal has regained its original weight before repeating the dose. Even this process has been unsuccessful in our hands. Without going into further details we will give the rule that we have adopted and which invariably leads to the successful immunization of the rabbit. First, the primary dose of eel serum should never exceed one twenty-five-hundredth of a cubic centimeter, and the injection should be made under the skin. The second dose should be the same amount as the first (one twenty-five-hundredth of a cubic centimeter), but must not be given in a shorter interval than ten days after the first injection. Second, the second injection can be given whether the animal has regained its original weight or not. Third, injections following the second can be given every day, under the skin, intravenous, or in the peritoneum and each succeeding dose augmented until one cubic centimeter is borne without inconvenience or accident.

These injections must be continued until a total of at least six or eight cubic centimeters of eel serum have been injected, when the animal can be said to be immunized. To obtain the serum of this vaccinated animal it can be bled twenty-four hours to twelve days after the last injection of eel serum. It may be stated that during the course of this immunization there usually appears, after the fifth or sixth injection, at the point of inoculation, an abscess filled with thick sterile pus.

THE SERUM OF A RABBIT IMMUNIZED WITH EEL SERUM. — In the study of the serum of a rabbit immunized with eel serum the following points must be considered: First, the total quantity of eel serum injected. Second, interval of time allowed to elapse between the last injection and taking the vaccinated rabbit's blood; third, power, *in vitro*, of the immunized rabbit's serum to protect the blood globules of a normal rabbit against the dissolving action of eel serum; fourth, power, *in vivo*, to protect a normal rabbit against the toxic action of eel serum; fifth, the production of a precipitate in eel serum upon the addition of the serum of an immunized rabbit.

Tchistovitch (loc. cit.) says that it is only necessary to "make two to four injections of a small dose of eel serum in order to provoke antitoxic properties. . . . Wishing to increase the antitoxic property, we chose a series of rabbits into which we injected increasing doses of eel serum and tested from time to time the antitoxic power of their blood; we observed a rather unlooked-for fact — the antitoxine progressively diminished during the course of the immunization."

The results of our experiments have been quite different. Rabbits that we have given two to four injections of a small dose of eel serum have furnished a serum that gives a cloudiness and ultimately a precipitate more or less dense when mixed with eel serum; and possesses in a high degree the power of protecting *in vitro*, the red globules of a normal rabbit from the solvent action of eel serum; but *in vivo*, even ten cubic centimeters of this serum has not succeeded in protecting a normal rabbit against the poisonous action of

one-tenth of a cubic centimeter of eel serum. Lastly, we have not found that the antitoxine has "progressively diminished," but upon the contrary, the antitoxic power has always been in proportion to the total quantity of eel serum injected. Which means that the larger the total quantity of eel serum injected into the animal, proportionately the stronger was the antitoxine produced.

Some experimenters insist that it is necessary the rabbit should be bled not later than twenty-four hours after the last injection, in order to prevent a diminution of antitoxine. In the table below we establish a comparison between a serum obtained several days, and another twenty-four hours after the last injection. In order to determine the antitoxic power of the serum *in vivo* we have injected two cubic centimeters in the marginal vein of a normal rabbit's ear; this is followed three hours after by the administration of two-tenths of a cubic centimeter (surely fatal dose) of fresh serum of the eel in the vein of the other ear. At this time an intravenous injection of one-tenth of a cubic centimeter of the same eel serum was made in the ear of a normal rabbit; the result of this latter part of the experiment has invariably ended in the death of the control animal, thus proving the toxicity of the eel serum employed.

TABLE I.

Mark of animal.	Largest dose injected at one time.	Total am. of eel serum.	Time of bleeding after the last injection.	Power to protect <i>in vitro</i> normal red globules.	Precipitate when added to eel serum.	Protection <i>in vivo</i> .	Mark of serum.
I.	0.30 c.c.	2.00 c.c.	24 hours.	Protects.	Light.	<i>Will not protect</i> even in the proportions of 1 to 100.	A
II.	0.30 c.c.	2.00 c.c.	12 days.	Protects.	Very heavy.	<i>Will not protect</i> even in the proportions of 1 to 100.	D
III.	0.50 c.c.	3.50 c.c.	24 hours.	Protects.	Light.	<i>Will not protect</i> even in the proportions of 1 to 100.	C
IV.	0.70 c.c.	4.00 c.c.	24 hours.	Protects.	Heavy.	<i>Will not protect</i> even in the proportions of 1 to 100.	F
V.	1.00 c.c.	8.00 c.c.	24 hours.	Protects.	Very heavy.	<i>Protects</i> in the proportions of 1 to 4.	E

While testing serum E we observed that it was highly antitoxic, for when two cubic centimeters of this serum were injected into the marginal vein of a normal rabbit's ear, three hours after one-half of a cubic centimeter of fresh eel serum could be injected into the marginal vein of the other ear, without the least effect. Three months after this experiment the animal was in good condition, thus demonstrating that both the immediate and remote effects of the poison were neutralized.

We have often observed every detail of the physiological action of ichthyotoxin and have nothing to add to descriptions already given by others except to note that the antitoxic serum prevents the contraction of the pupil. We wish to

call attention to this particular symptom, for it was the subject of a series of experiments performed by Camus and Gley, wherein they endeavored to prevent contraction of the pupil by instillation in the eye and injection under the skin of Atropin solutions, but their results were uniformly negative.

Inasmuch as it is well known that certain morphological elements have the power to neutralize or fix certain toxines, for example, the fixation of tetanus toxine by nervous tissue, etc., we undertook to saturate eel serum with a mass of red globules of a normal rabbit.* To this end we treated one cubic centimeter of fresh eel serum with successive quantities of normal rabbit blood globules until the last portion remained undissolved, and injected the mixture into the marginal vein of a normal rabbit; the only effect that followed the injection of this enormous dose was a contraction of the pupil upon the side the injection was made; the other pupil remained normal.

Bordet, in his hemolytic reaction, has proved the presence of a sensibilizing substance (*substance sensibilisatrice*) in the serum of an animal injected with the blood of a different species of animal; we have endeavored to find a similar body in the serum of rabbits vaccinated with eel blood. As a first step in this reaction, we proved that the serum of a rabbit that had been vaccinated with eel blood would easily dissolve the red globules of the eel. Now, upon heating this serum to fifty-five and eight-tenths degrees (Cent.) during one hour, to destroy the alexine, and mixing it with the blood globules of the eel and a small quantity of normal rabbit serum, no solution took place. This result has been repeatedly confirmed in a great number of our experiments.

We have also observed that the blood globules (freed from every trace of serum by repeated washings with physiological salt solution) of rabbits that have been vaccinated against the serum of eels, offer more or less resistance to the hemolytic action of this latter substance, but we have never

*The blood globules of a rabbit vaccinated with eel serum will not fix or neutralize eel serum *in vitro*.

succeeded in obtaining a complete resistance, even by the globules of a rabbit which had received a total of twenty cubic centimeters of eel serum.

SERUM OF A RABBIT THAT HAS BEEN VACCINATED WITH THE BLOOD GLOBULES OF THE EEL. — Until now we have occupied ourselves with the serum of rabbits that have been vaccinated with eel serum; but in this part we wish to give the results of our study upon the serum of rabbits that have been vaccinated with the blood globules of the eel.

The blood globules of the eel, freed from serum by repeated washings, possess no toxic action, whether injected into the vein, under the skin, or in the peritoneum of a rabbit, even in very large doses.

To prove this we have injected the well washed globules from twenty cubic centimeters of eel blood into the vein of a rabbit without causing the least immediate or remote effect. To vaccinate a rabbit with the blood globules of the eel we have made three or four intraperitoneal injections, at an interval of about seven days between each injection, of the globules suspended in artificial serum, provided by five cubic centimeters of eel blood; seven days after the last injection the vaccinated animal furnished a serum that produced a heavy precipitate when mixed with eel serum, and instead of dissolving the blood globules of the eel, strongly agglutinated them. In addition to this, it protected, *in vitro*, the blood globules of a normal rabbit from the hemolytic action of eel serum, in the proportions of five parts of vaccinated rabbit serum to one part of eel serum. This serum protects, *in vivo*, in the proportions of twenty-five to one.

The blood globules of a rabbit that has been vaccinated with the blood globules of the eel resist in a much higher degree the hemolytic action of eel serum than those of rabbits vaccinated with eel serum.

THE SERUM OF AN EEL THAT HAS BEEN VACCINATED WITH RABBIT SERUM. — According to all modern theories relative to the intimate mechanism of the production of anti-

corps, under which name are included the antitoxines, fixateurs (sensibilizing bodies), agglutinines, and perhaps the precipitines, the credit is given to one or more of the different kinds of white cells that circulate in the blood. To illustrate this point still further we can take, for example, the vaccination of a horse with diphtheria toxins and the subsequent production in the horse's organism of an anticorp, the diphtheria antitoxine. This process can be repeated by using other toxins for the production of their corresponding antitoxines and in animals other than the horse, that is to say, different species of warm-blooded animals; for up to the present, experimenters have not been able to observe the production of an antitoxine in a cold-blooded animal that has been vaccinated with a toxin, in spite of the fact that many members of this latter class of animals possess the same varieties of white cells as warm-blooded animals. If a cold-blooded animal like an eel or a frog be inoculated with a bacterial toxin, no antitoxine is formed; but inasmuch as an antitoxine is only one member of the class anticorp, we have undertaken to excite in an animal of the cold-blooded type the production of an anticorp by the injection of eels with, in the first series, rabbit serum, and in the second, the red globules of a rabbit.

In our experiments we have injected the eels, at intervals of six days, with two to four cubic centimeters of normal rabbit serum, these injections being made under the skin and in the muscles. After six injections the eel was bled. The resulting serum was of a yellowish color, and when added to normal rabbit serum produced a precipitate. This precipitate would be formed more rapidly and much thicker if, instead of normal rabbit serum the serum of a rabbit that had been vaccinated with the serum or blood globules of the eel was substituted. When mixed with the blood globules of a normal rabbit it dissolves them instantly, but does not protect the blood globules of the normal eel against the hemolytic action of the serum of a rabbit that has been vaccinated with eel serum or blood globules. The blood globules of an eel that has been vaccinated with rabbit serum are a little

more resistant to the hemolytic action of the serum of a vaccinated rabbit than those of the normal eel. Lastly this serum is as toxic as normal eel serum.

SERUM OF THE EEL THAT HAS BEEN VACCINATED WITH NORMAL RABBIT BLOOD GLOBULES. — When the eel is vaccinated with the blood globules (freed from every trace of serum by repeated washings with physiological salt solution) of the rabbit, it furnishes a serum quite different from that given by eels vaccinated with rabbit serum. We have injected a series of eels with these globules, from ten cubic centimeters of rabbit blood, at intervals of six days between each injection; six days after the last injection, the eels have given a serum, no longer yellow or bluish as before, but distinctly red (auto-hemolytic). It produces a thick precipitate when mixed with normal rabbit serum. It does not dissolve the red globules of the normal rabbit, but protects those of the normal eel against the hemolytic action of the serum of a rabbit that has been vaccinated either with eel serum or globules. In this property we have the positive manifestation of the presence of an anticorp, antihemolysin. This serum is extremely toxic, almost four times more toxic than normal eel serum. The serum of normal or vaccinated rabbits does not dissolve the blood globules of an eel that has been vaccinated with the blood globules of the rabbit.

CONCLUSIONS.

First. Eel serum is extremely poisonous when injected into the veins of the rabbit or guinea-pig.

Second. There is an immediate and a remote effect following the injection of a poisonous dose of eel serum. Rabbits resist the latter effect much better than the former, whereas with guinea-pigs the reverse is true.

Third. The immunization of the guinea-pig with eel serum has not yet been attained. That of the rabbit, although a delicate task, is usually successful.

Fourth. The serum of a rabbit that has been vaccinated

with eel serum possesses antitoxic properties that neutralize, *in vitro* and *in vivo*, the poisonous action of eel serum.

Fifth. Eel serum has the property of dissolving the blood globules of the normal rabbit. The resistance of the blood globules of a rabbit that has been vaccinated with eel serum is somewhat above that offered by the blood globules of a normal rabbit.

Sixth. The blood globules of a normal rabbit have the power to fix upon themselves the poisonous principle in eel serum and neutralize its effect. This property is wanting in the blood globules of a rabbit that has been vaccinated with eel serum or eel blood globules. This is one fact in the evidence going to prove that the antitoxine is produced by the red corpuscles of the rabbit.

Seventh. A rabbit vaccinated with the blood globules of the eel produces a serum that gives a heavier precipitate, when mixed with eel serum, than the serum of a rabbit that has been vaccinated with eel serum.

Eighth. The serum of a rabbit that has been vaccinated with the serum or blood globules of the eel has the power to dissolve, *in vitro*, the blood globules of the eel. Now when an eel has been vaccinated with the serum or the blood globules of a rabbit, the serum of this vaccinated eel will protect, *in vitro*, the normal eel's blood globules against the dissolving action of the hemolytic rabbit serum. Hence the conclusion must be drawn that the organism of the eel has been excited by this vaccination to produce an antitoxine. That is to say, an anticytotoxine that is capable of neutralizing the cytotoxines existing in the serum of the vaccinated rabbit.

Ninth. The mechanism of a hemolytic reaction, according to Bordet, depends upon the conjunction of two bodies — the sensitizing body (*substance sensibilisatrice*) and the alexine; but we have found that the former body does not exist in the serum of a rabbit that has been vaccinated with eel blood, or that it is destroyed at a temperature of fifty-five and eight-tenths degrees (Cent.), and in this respect does not agree with the generally accepted property of the "substance sensibilisatrice."

Tenth. Therefore, inasmuch as the red globules of a rabbit vaccinated with the serum or the blood globules of an eel offer a higher degree of resistance to the dissolving action of eel serum than do the blood globules of the normal rabbit, and the inability of the blood globules of a rabbit that has been vaccinated with the serum or the blood globules of the eel, to neutralize the toxic principle in eel serum, together with the non-production of the sensibilizing body, we conclude that eel blood, when injected into the organism of the rabbit, excites the red globules of that animal to produce an antitoxine.

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DOG'S BLOOD. — DIFFERENTIAL COUNTS OF LEUCOCYTES.

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The comparative morphology of the white corpuscles in the blood has been studied in a number of animals used for experimental purposes. The differential numerical relations of the leucocytes has been estimated in a few. This has been done for rabbit's blood by Brinckerhoff and Tyzzer¹ and for dog's blood by Dawson² and by Tallqvist and Von Willebrand.³ Believing that further work along similar lines would still be of value, the writers feel justified in adding another series of counts to those which have already been made.

The present series includes differential counts of the leucocytes in the blood of twenty dogs, all apparently normal, so far as could be ascertained, in many instances, by post-mortem examination. In the majority of cases, counts of the red corpuscles, and in all cases, of the leucocytes were made. The blood was taken at least twelve hours after feeding. The blood smears were stained by Ehrlich's triple stain; by eosin and methylene blue; by Jenner's stain; by Leischman's stain; by Unna's polychrome-methylene blue; and by Dahlia for mast cells.

As a rule the counts of the leucocytes, stained by the various methods, agreed very closely. The table, therefore, represents an average of the counts made from the various stains.

It may be of value to mention a few points in the technic of staining by the various methods. With eosin-methylene blue the results are not uniform. Usually the nuclei and granules alone are stained; occasionally the cell body also. When this occurs the results are as good as those obtained by any other stain. Much depends on the fixation of the

¹ Journal of Medical Research, Vol. 7, No. 4.

² American Journal of Physiology, Vol. 4, No. 1.

³ Skand. Archiv fur Physiologie, 1899, ix, p. 37.

smear, which is preferably done by alcohol and ether. With Ehrlich's triple stain the nucleus can be better differentiated by treating the stained and dried smear, from one to three seconds, with a saturated aqueous solution of methylene blue as used by Hewes.¹ With Jenner's stain,² the results depend largely on the care exercised in washing and drying. The smear, which has been dried in the air (no previous fixation required) should be flooded with the stain for about three minutes and then washed by gentle agitation in a dish of distilled water until the specimen has attained a pink hue. Tap water should not be used. After washing, the smear should be allowed to dry in the air or by gentle warming. Blotting paper should not be applied. Leischman's method³ gave uniform and satisfactory results. The application of this stain is, briefly, as follows: To the dried smear three or four drops of the stain are added and evenly distributed over the blood film. After about half a minute the stain on the cover-slip is diluted with six or eight drops of distilled water. After staining about five minutes, in the case of thin films, the stain is gently washed off in distilled water and the water allowed to remain on the film for a minute longer. The smear is then ready for examination, either directly in water or after drying (without heat) and mounting in xylol balsam. With this stain the nuclei of the colorless corpuscles are sharply differentiated, taking a deep ruby red, while the extranuclear protoplasm remains unstained, with the exception of the mononuclear forms which may take a pale blue tint.

The leucocytes have been classified under the following headings:

Polymorphonuclear, lymphocytes or small mononuclear, large mononuclear, eosinophiles and mast cells.

DESCRIPTION OF CORPUSCLES AS STAINED BY THE VARIOUS METHODS. — With eosin and methylene blue, the nuclei

¹ Journal of the Boston Soc. of Med. Sciences, Vol. II, p. 70.

² Lancet, 1899, Vol. I.

³ Leischman. A simple and rapid method of producing Romanowsky staining in malarial and other blood films. British Med. Journ., 1901, Vol. II, p. 757.

(Modified by James H. Wright. The Journal of Medical Research, Vol. VII, p. 138, 1902. — Editor.)

of the polymorphonuclear leucocytes and of the lymphocytes stain a deep sky blue, and of the large monuclear forms, a lighter blue. Eosinophile granules stain bright red. The cytoplasm either does not show at all or is tinged a faint pink. With Ehrlich's triple stain, the nuclei of the polynuclear forms take a dark blue or a blue-black, of the large mononuclear forms a light blue, and of the eosinophiles a still lighter blue. The eosinophile granules are stained a purple red. The cell body is stained a reddish brown. With Jenner's stain, the nuclei of all the forms are stained a light blue and the cytoplasm a light pink. The eosinophile granules take a bright red. With Leischman's method, the nuclei are stained ruby red and the cell bodies a faint terra-cotta or remain colorless.

FORMS OF LEUCOCYTES.¹ — The polymorphonuclear leucocyte is the most frequent form found in dog's blood. The cell, in cover-glass preparations, is generally round and about twice the size of a red blood corpuscle. There is, however, considerable variation in the size of the cell body. The most common form of nucleus is that of a partial coil or twist without separation into unconnected nuclear masses, although this apparent form of nucleus also occurs. The nuclear chromatin has a frequent tendency to a mural arrangement as described by Brinckerhoff and Tyzzer for rabbit's blood. This is especially noticeable where there are apparently several separate nuclear masses. The cytoplasm is most commonly homogeneous. Often, however, it has a granular appearance and occasionally contains a varying number of very fine reddish granules resembling the neutrophile granules of the polynuclear leucocyte in man's blood. The relative number of fine granular leucocytes varies in different specimens of dog's blood. In all specimens which we have examined, the homogeneous variety has far outnumbered the fine granular variety. This is somewhat at variance with the observations of Hirschfeld,² who has found the

¹ See also Dawson (loc. cit.), who has given in some detail the morphology of the leucocytes in dog's blood.

² Virchow's Archives, Bd. 149.

granular variety more frequently. We have classified the homogeneous and fine granular variety together in our table of differential counts. The lymphocyte or small mononuclear cell comes next in point of number to the polynuclear. There is a great variation in the size of this cell as compared with the one of the preceding group, and there are many transitional forms between this and the large mononuclear. The typical cell of this variety is about the size of or slightly larger than a red blood corpuscle and contains a rounded nucleus which almost completely fills the cell. The nucleus is, as a rule, solid (Plate XIX., Fig. 2). Frequently, the chromatin is murally arranged in the form of a ring (Plate XIX., Fig. 4). Occasionally it has the appearance of segmentation (Plate XIX., Fig. 3). Forms are also sometimes seen with a double nucleus as if in the act of division, although we have not seen mytotic figures. The large mononuclear leucocyte is more variable in number. In this class we have included only those leucocytes with a single round or bean-shaped nucleus and considerable cytoplasm (Plate XIX., Fig. 5). The nucleus is most commonly bean or kidney shaped and takes a lighter shade of stain than the small mononuclear. We have several times seen small granules, like neutrophile granules, in the cytoplasm of this form. The cytoplasm is, with these few exceptions, homogeneous or may show an indistinct network. We have seen forms with a very distinct network, but have ascribed this, possibly, to the method of fixing. Between this and the lymphocyte and also between the mononuclear form and the polynuclear variety there are transitional forms which it is difficult to classify. The eosinophile (Plate XIX., Fig. 8) is a corpuscle usually larger than the polynuclear form, with a nucleus that takes a lighter stain and is not so well differentiated. The granules, which are large, vary much in form and size as described by Hirschfeld (loc. cit.). They are usually round, but may be oval, bacillus shaped, or irregular in form. The number of granules varies also. Some cells are packed with granules, the granules having the appearance of crowding and partially hiding the nucleus, while other cells contain only a few. Between this cell and the polymorphonuclear leucocyte there are also a few

transitional forms both in respect to the size of the granules and the form of the nucleus. Mast cells were seen so infrequently, although very careful search was made for them, that they have not been included in the table of differential counts. This cell, when found, is about the size of the polymorphonuclear leucocyte. The nucleus is faint and poor in chromatin. The granules are fine and metachromatic (Plate XIX., Fig. 9).

TABLE OF DIFFERENTIAL COUNTS.

	Polymorpho- nuclear. Per cent.	Lymphocytes. Per cent.	Large mono- nuclear. Per cent.	Eosinophiles. Per cent.
Dog 1	67.7	24.7	5.3	2.1
" 2	57.1	29.3	8.8	4.6
" 3	64.6	25.1	9.0	1.1
" 4	74.5	9.7	9.7	1.4
" 5	87.5	3.7	7.7	1.0
" 6	64.5	21.3	6.8	7.2
" 7	67.0	16.5	13.2	3.0
" 8	60.0	28.3	4.2	6.2
" 9	66.6	23.0	8.0	2.2
" 10	69.8	16.5	5.1	8.4
" 11	60.1	22.8	3.7	15.2
" 12	54.3	35.3	3.9	4.9
" 13	66.2	21.8	4.8	6.9
" 14	73.0	14.6	7.2	5.1
" 15	63.9	21.2	8.4	6.7
" 16	66.9	19.8	7.7	5.4
" 17	59.8	35.0	4.2	0.8
" 18	73.1	15.9	6.0	5.1
" 19	61.7	28.3	7.0	2.7
" 20	54.9	26.5	4.9	13.6
Average	65.7	21.0	6.8	5.3

The maximal number of red corpuscles per cubic millimeter of blood was 8,030,000; the minimal number was 4,225,000. The maximal leucocyte count was 14,375; the minimal was 7,200. The average number of reds was 6,206,000 and of leucocytes 9,526 per cubic millimeter.

The following results of differential counts have been obtained:

In ten dogs, by Dawson (loc. cit.):

Polymorphonuclear leucocytes	.62-4-65	per cent	average	64.56	per cent.
Lymphocytes	11.2-31.6	"	"	22.17	"
Oxyphilic leucocytes	2.6-21.6	"	"	8.85	"
Other forms	1.2-9.4	"	"	4.42	"

Tallqvist and Willebrand (loc. cit.) have tabulated differential counts in fifteen dogs as follows:

Polymorphonuclear leucocytes	.68-4-75.6	per cent	average	70-80	per cent.
Lymphocytes	4.2-10.8	"	"	5-10	"
Oxyphils	0.2-6.6	"	"	4-8	"
Other forms	9.6-17.4	"	"	10-15	"

SUMMARY.—I. There are five distinct types of leucocytes in circulating dog's blood. These are: a small mononuclear leucocyte, a large mononuclear form, a polymorphonuclear form, usually non-granular but occasionally with fine neutrophile granules, and eosinophiles with coarse round or oval granules, and a mast cell with fine metachromatic granules.

II. The average percentage of the polymorphonuclear form is sixty-five and seven-tenths; of the small mononuclear, twenty-one; of the large mononuclear, six and eight-tenths; of the eosinophile, five and three-tenths. The occurrence of the mast cell is rare.

III. The percentage number of the polymorphonuclear form is subject to the least variation and that of the eosinophile to the most variation.

IV. In the few cases of high polymorphonuclear counts, the number of the lymphocytes was correspondingly de-

creased. On the other hand, where the eosinophile percentage was high, the percentage of other polymorphonuclear forms was low.

EXPLANATION OF PLATE XIX.

- Figure 1. Red corpuscles for comparison of size.
- Figure 2. Lymphocyte or small mononuclear leucocyte with even distribution of chromatin.
- Figure 3. Lymphocyte with segmental arrangement of chromatin.
- Figure 4. Lymphocyte with mural arrangement of chromatin, giving appearance of a ring.
- Figure 5. Large mononuclear form with bean-shaped nucleus poor in chromatin.
- Figure 6. Polymorphonuclear form without granules.
- Figure 7. Polymorphonuclear form with fine granules.
- Figure 8. Eosinophile; nucleus poor in chromatin.
- Figure 9. Mast cell; nucleus barely visible.

ON BRANCHING FORMS OF CERTAIN BACTERIA.

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Considerable discussion has occurred among bacteriologists concerning the morphology of certain bacteria and the places to which these bacteria must be assigned in the class of fungi. The bacteria which have excited most interest are the bacilli of tuberculosis and of diphtheria. Several other organisms have been described as occasionally producing branching forms, although not nearly with such frequency as those before mentioned.

The *Bacillus ozenae*, ordinarily a very short, thin organism, was found by Hugo Marx,¹ Levy, and Semmer to be at times much elongated (10–26 μ) and to have branches fifteen microns in length. He therefore classes this organism with the streptothriceæ of Kruse, believing true branching sufficient to distinguish it from the bacteria. The anomalous form occurred chiefly in old cultures.

The bacillus of swine erysipelas was described by Kitt² as producing branches in old bouillon cultures. Kitt later³ found that his culture had contained a "streptothrix" intimately mixed with the bacillus of swine erysipelas. In his earlier paper, however, he states that Lorenz had obtained branching forms of the same organism in 1892 by allowing it to grow in bouillon, which had contained bacteria of swine pest (*Schweineseuchebakterien*), which were killed by heat and removed by filtration.

Many shapes and forms of *Bacillus coli communis* have been found under varying conditions, notably coccoid and diplococcoid varieties.⁴ Recently branching has been dis-

¹ *Centralbl. f. Bakt.*, 1899, Vol. 25, pp. 274–8.

² *Cent. f. Bakt.*, 1897, Vol. 22, pp. 726–732.

³ *Ibid.*, Vol. 23, p. 601.

⁴ Adami, Abbott, and Nicholson, *Jour. Exp. Med.*, 1899, Vol. 4, Nos. 3 and 4.

covered by Ohlmacher¹ in cultures of this organism from a case of septicemia. The branching forms, also organisms with metachromatic granules, appeared in one of the early inoculations from the heart's blood.

Budding, but not true branching, of *Streptococcus pyogenes* and *Pneumococcus* have been described by Babes² as occurring under unusual circumstances.

A "typhoid like" bacillus, *Bacterium aceti* and bacteria from the root of *vicia villosa* and of *lupinus albus* are pictured by A. Fischer³ as forming true branches or undergoing dichotomous division. In these instances branching occurred when the organisms were allowed to multiply under unfavorable conditions, *e.g.*, ammonium chloride, glucose, or other substances were added to the media, or the tubes were placed in an incubator kept too warm for the most favorable development of the bacteria.

The branching of *B. diphtheriæ* and *B. tuberculosis* has been most thoroughly exploited. In this country Hill⁴ has found branching of the diphtheria bacillus to be very frequent and was even able to trace under the microscope the mechanism of its production. In the case of this organism branching occurred very early — within twenty-four hours — on ordinary media, a fact quite out of accord with the findings in the case of other bacteria. It is difficult to explain the branching of this bacillus on the same grounds as apply elsewhere.

Tubercle bacilli and their peculiar morphological variations have been discussed at great length since the early work of Babes and Cornil.⁵ It is true that in their paper no mention is made of the formation of branches, but the illustrations accompanying the text show organisms which may or may not be branching. Babes did not till many years later describe these plates as illustrating the branching

¹ Jour. Med. Research, 1902, Vol. 7, No. 1, pp. 128-136.

² Zeit. f. Hyg. u. Infekt., 1895, Vol. 20, pp. 412-437.

³ Vorlesungen über Bakterien, Jena, 1897.

⁴ Jour. of Med. Research, Vol. vii, p. 115, and Annual Report of the Boston Board of Health, 1901.

⁵ Journal d'Anat., 1883, Vol. 19, pp. 456-480.

of tubercle bacilli.¹ To Petrone² properly belongs credit for the first thorough description of this phenomenon. Metchnikoff³ discovered branching in tubercle bacilli, when these were grown at 43.6° C. Inasmuch as human tubercle bacilli do not multiply at all at this temperature, it is probable that he employed the avian variety, which at that time was considered identical with the human. Klein,⁴ Dixon,⁵ Coppen Jones,⁶ Fischel,⁷ Schulze,⁸ Bruns,⁹ Domec,¹⁰ and others have also written extensively on the finding of branching forms. A thorough review of this literature and the relations of the ray fungi to tubercle bacilli has been published by Hektoen.¹¹ It is of some significance that practically all of the branching forms noted were discovered in old cultures. In most cases the tubes had been allowed to remain in the incubator at the ordinary temperature for many weeks or months. Sometimes the media are described as having evaporated to a considerable extent. In a few instances the age of the culture and the nature of the medium are not mentioned. It is also noteworthy that most observers have found that cultures containing such bacteria were much less markedly pathogenic than the ordinary tubercle bacilli.

Because of the occasional occurrence of branching, it is usually assumed that these organisms belong to the hyphomycetæ. Lubarsch¹² tabulates the characteristics of this group as follows:

1. They can form in the animal body radiating foci with club-shaped prolongations.

¹ Loc. cit.

² Cited by Coppen Jones. C. f. Bakt., Vol. 20, p. 393.

³ Virchow's Archiv, 1888, Vol. 113, pp. 63-94.

⁴ C. f. Bakt., 1890, Vol. 7, No. 25, p. 712.

⁵ Medical News, Oct. 19, 1889.

⁶ C. f. Bakt., 1895, Vol. 17, No. 1, p. 1.

⁷ Fortschritte der Medicin, 1892, Vol. 10, No. 22, p. 908.

⁸ Z. f. Hyg. u. Inf., 1899, Vol. 31, p. 153.

⁹ C. f. Bakt., 1895, Vol. 17, No. 23.

¹⁰ Arch. de med. exp. et d'anat. path., 1892, Vol. 4, p. 105.

¹¹ New York Medical Journal, May 26, 1900. See also Abbot and Gildersleeve, Univ. of Fenn. Med. Bull., June, 1902.

¹² Z. f. Hyg. u. Inf., Vol. 31, pp. 187-220.

2. The threads have a tendency to break up into rods, balls, or spirals.

3. In cultures, greater or smaller club-like swellings of the threads or rods may occur.

4. Colonies in artificial media are firmly attached to the medium and are crumbly in character; they tend to produce yellow or red colors.

5. When they produce disease, they tend to produce tubercles in the animal body.

While Lubarsch does not class the *B. tuberculosis* along with actinomyces, he places them with the streptothriceæ, which in turn he classes under the hyphomycetæ. The usual appearances of the tubercle bacilli are usually attributed to growth in unfavorable surroundings, the branching forms are thought to be a reversion to an original saprophytic species, from which the tubercle bacilli are supposed to have sprung. Klein believes that the forms ordinarily seen are only a phase in the life of a microorganism morphologically related to the hyphomycetæ. Coppen Jones¹ suggests for them the name "tuberculomyces," Metchnikoff "schlerothrix Kochii."

Craig² has described branching bacilli, both acid and alcohol proof, occurring in the sputum of a case of pulmonary gangrene, apparently tuberculous in nature. These he assumes to be tubercle bacilli both on account of the clinical features and because of the reaction to aniline dyes. There is no statement in his paper as to cultural work, experimental inoculation of animals, or necropsy. Similar findings have been recorded of late by Fraenkel, Rabinowitch, and others.³ In cases in which cultures have been made, a branching organism has been isolated, which is nonpathogenic to lower animals. It appears doubtful whether Craig was not dealing with this organism and not with the tubercle bacillus as he supposed. The finding of tubercle bacilli, which were branched, in the tissues or sputum has been ex-

¹ C. f. Bakt., Vol. 20, p. 393.

² Jour. Exp. Med., 1898, Vol. 3, p. 363.

³ For bibliography, etc., see Ophüls, Jour. Med. Res., 1902, Vol. 8, No. 1: "Acid-proof bacilli in five cases of pulmonary gangrene."

tremely rare, so much so that it is doubtful whether they really ever occur spontaneously in the animal body in this form.

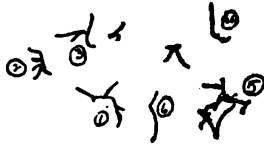


FIG. 1.

1. 29.4 μ . Branches, 12 μ and 4 μ .
2. 14 μ . Branches, 6 μ , 6 μ and 4 μ .
3. 16 μ . Branches, 14 μ and 4 μ .
4. 18 μ .
5. 18 μ without bends, etc.
6. 22 μ .

Tubercle bacilli from third generation on bouillon, 18 days old.



FIG. 2.

Typhoid bacilli grown for 18 hours in 3 per cent bouillon. Inoculation made from 2 per cent bouillon in which they had been allowed to multiply for 24 hours. Very similar figures were found in 4 per cent bouillon as well. Many bacilli were 15 μ in length. Great variation is seen in the intensity of the stain. Figures 4 and 5 are from older and more concentrated (4 per cent) cultures and show "metachromatic" granules and bipolar staining respectively.

It has been my good fortune to obtain very marked examples of branching forms of tubercle bacilli in bouillon cultures. The bouillon contained one per cent of peptone and six per cent of glycerine. The most extensive branching occurred in the fourth generation in bouillon after two weeks of growth at incubator temperature (37°-39° C.). In this instance, contrary to my usual method, I had accidentally failed to seal the cotton stopper of the tube with paraffin, so that the medium had evaporated nearly to one-half of its original amount. It was not possible to obtain subcultures from this medium. Two cubic centimeters, injected into the peritoneum of a guinea-pig, produced death from miliary

tuberculosis in seventeen days. The bacilli, isolated from the liver and spleen of the animal on Dorset's medium (mixed white and yolk of egg), had no morphological characteristics in common with the branching forms except the quality of being acid-proof.

The rapidity of the appearance of branching figures (two weeks) led me to consider the factors which might have induced it. Although I had met with branching forms before with some frequency, they had never been seen in such abundance or so soon after inoculation. The medium was identical with that in constant use; the sole difference lay in the degree of concentration. The remarkable variations in morphology resulting from changes in the concentration of the inorganic salts contained in the culture media have been studied by Teisi Matzuschita.¹ The appearance of bacilli in coccoid forms and even more startling phenomena are not unusual. Especial attention has been directed to the bacillus of bubonic plague on account of the value of this procedure in the diagnosis of the organism.² Branching had not been described, however, as a result of increasing the amount of salts in the media except by Fischer, whose work has been briefly mentioned. The fact that branching, too, might be a result of involution has been suggested, so far as noted, only by Fischer and Dixon. I attempted to produce similar forms rapidly by changing the concentration of the culture media. Tubercle bacilli were transplanted from potato tubes, whose bulbs contained six per cent aqueous glycerine, to bouillon tubes containing one per cent of peptone and common salt ranging from one-tenth to eight per cent. After transplantation, the tubes were sealed with paraffin so that no change could occur in the concentration of the contained salt. The level of the bouillon was marked on each tube, so that, in case evaporation occurred, it might be readily noted. The cultures were then kept at 38–39° C. for two weeks. In the attempt in this way to obtain branching figures, I was only moderately successful. The more concentrated media

¹ *Z. f. Hyg. u. Inf.*, 1900, Vol. 35, pp. 495–510.

² Hankin and Leumann. *C. f. Bakt.*, 1897, Vol. 22, pp. 438–440. Wilson, *Jour. Med. Research*, 1901, Vol. 1, pp. 53–58.

(4½ per cent to 8 per cent NaCl) did not permit the multiplication or growth of the bacilli. In the weaker solutions (½ to 2 per cent NaCl) no differences were noted from the ordinary appearances. In those of a moderate grade of concentration (2 to 4 per cent), branching forms were encountered quite frequently, but the multiplication of the bacilli was so slight that the experiments were abandoned and it was decided to attempt similar measures in the case of a more rapidly developing organism.

Results similar to the above were obtained also with a two per cent solution of Magnesium chloride.

The typhoid bacillus was chosen for these purposes, because, so far as known, branching forms had not been discovered and no reason had ever been suggested why it should be regarded as anything but a typical member of the schizomycetes. In the case of the typhoid bacillus, the same technic was employed, except that the cultures were examined as soon as the bouillon became cloudy—usually in from eighteen to thirty-six hours. No growth took place in tubes containing more than four and one-half per cent sodic chloride. In cultures containing two per cent sodic chloride at the end of twenty-four to thirty-six hours a few branching forms were noted. The bacilli were usually at least twice, often four or five times their usual length. The branches were often as long as the body of the bacillus. Frequently the typical appearance of dichotomous division was noted. The bacilli were but slightly motile. Their protoplasm seemed to vary in density in parts: in places taking the stain intensely, in other parts very faintly. It was often necessary to stain one or two smears to find a single branching form. In older cultures of the same concentration, the bacilli were usually shorter, often the staining was bipolar. More concentrated solutions of sodic chloride (2½–4½ per cent) early showed practically the same appearances; in addition were found in a few instances just such metachromatic granules as are often seen in diphtheria and tubercle bacilli and have been interpreted by some, notably Babes, as spores. The bacilli were non-motile. At the end of thirty-six to forty-

eight hours nothing but fragments of bacilli were found in the media of higher concentration; complete bacteriolysis had taken place.

For these experiments two cultures of typhoid bacilli were used:

(a.) Obtained from the gall bladder in a case of typhoid fever; it agglutinated rapidly with blood serum from typhoid patients; age on artificial media about one month.

(b.) Obtained from Chicago Board of Health; age unknown.

It is difficult to explain the mechanism of the formation of branches. Fischer¹ observed the rupture of the membrane of bacteria as a result of changes in the osmotic pressure of the media surrounding them, and the extrusion of portions of the protoplasm, "plasmoptysis" (spitting of protoplasm). It is probable that, after restoration of osmotic equilibrium, a new membrane is formed to include the extruded protoplasm. If complete plasmolysis has occurred, further development of the organism must cease; otherwise life continues. It is possible that growth of the protoplasm may be especially vigorous at the point where the cell wall has been most weakened. If that is the case, the origin of branches is readily understood. Analogous results of injury have been noted frequently in many of the lower classes of animals. Whether the causes of branching in tubercle bacilli are of the same nature, cannot be said. Probably many other factors are concerned, such as the toxins and other products of the tubercle bacilli in the culture media, or the glycerine and glucose, so frequently used in media, may play a part. At any rate, the occurrence of branching tubercle bacilli in old cultures, their loss of pathogenicity and their slight multiplication, would all suggest that such forms are the result of degenerative changes rather than that they are a reversion to a higher type of fungus.

Lack of time has prevented the accomplishment of more complete experiments. It is desirable that the work be re-

¹ *Zeit. f. Hyg.*, 1900, Vol. 35, pp. 1-58.

peated and more be done along the same lines. It may be possible in this way to settle the much-mooted question of the proper classification of tubercle bacilli and other occasionally branching organisms.

(It is my pleasant duty to thank Professor Hektoen for many valuable suggestions and assistance in this work.)

A CHEMICAL STUDY OF THE LIVER FROM A CASE OF ACUTE
YELLOW ATROPHY OF THE LIVER.

ALONZO ENGLEBERT TAYLOR.

(From the Hearst Pathological Laboratory, University of California.)

The subject came to autopsy in the City and County Hospital from the wards of Prof. William Watt Kerr. The history was fairly typical, the diagnosis *intra vitam* had been confirmed by the demonstration in the urine of leucin and tyrosin in noteworthy quantities. The autopsy was performed six hours after death.

The liver was very much atrophied, and weighed nine hundred and ninety grams; the dimensions were reduced to less than one-half the normal. The color was a golden yellow, interspersed with areas of deep red. The right lobe was quite soft, the left lobe was notably cirrhotic. The kidneys presented an acute hemorrhagic nephritis. The tissues in general were deeply jaundiced. The gall bladder and bile ducts presented no signs of disease, nor did the pancreas.

Sections of the liver revealed the appearances usually associated with acute yellow atrophy of the liver. Upon a conservative estimate over three-quarters of the liver cells in the areas inspected were degenerated beyond cytological recognition.

The liver, with the exception of a strip weighing about ten grams, was cut under absolute alcohol into small pieces. Upon the following day the alcohol was changed. Upon the following day the tissue was extracted with ether. The alcoholic and etherial extracts were united, evaporated at 50° under one hundred millimeters pressure to dryness, the residue extracted with petroleum ether, this residue dried, extracted with water, and the final residue returned to the liver substance. The tissue was then dried, and ground to an impalpable powder, which was repeatedly extracted with hot water, and then with hot water acidulated with hydro-

chloric acid. To these united watery extracts was then added the watery extract of the residue after extraction with petroleum ether. Following the aqueous extraction the tissue was dried, and extracted for one hundred hours with ether in the Soxhlet apparatus. This ethereal extract was joined to the extract in petroleum ether. The material was thus divided into three parts:

- (a.) The dried residue.
- (b.) The watery extract.
- (c.) The ethereal extract, crude fat.

The sum of the substances in all three was 129.93 gms., which represents the dehydrated residue of the liver. The normal liver of the male has a dried residue varying from 350 to 450 gms., the percentage of the weight of the fresh liver ranging from 21 to 26 per cent. The residue in the present case was about one-third the normal. Since the percentage of solids was but 14.2+ %, it is apparent that the organ was notably hydremic, and this despite the cirrhotic condition of the left lobe. In the normal liver the connective tissue makes up probably about one-sixth of the solid tissue, or about sixty to seventy grams. Upon the basis of the microscopic appearances of the sections, it may be fairly inferred that the connective and vascular tissues had not suffered. Thus there would remain only some sixty grams of the solids as the residue of the liver cells — about one-fourth the normal quantity. These figures, though obviously only approximate, serve as a crude mathematical expression of the extent of the atrophy.

The ash of the liver was 9.55 per mille. The total nitrogen, determined by the Kjeldahl method, was 13.334 per cent of the residue, which figure approximates the normal percentage. The nitrogen in the watery extract was 0.603 g., in the crude fat 0.243. Added together we have 15.180 g. of nitrogen, as the total of the organ. This is about one-third of the usual nitrogen in a normal male liver.

The watery extract contained no glycogen, but did contain a small quantity of sugar, whose presence was confirmed by the fermentation test, though not estimated quantitatively.

The larger part of the watery extract was studied for the products of protein hydrolysis, in particular for hexon bases and monamido-acids. For the hexon bases the methods of Kossel and Kutscher were used, for the amido-acids, the new method of Fischer was employed. Heretofore it has not been technically possible to isolate and separate monamido-acids in animal material; this can now be easily and reliably accomplished by means of the Fischer method.

In the search for hexon bases negative results were obtained. This does not mean that histidin, arginin, and lysin do not appear in the degenerations of liver substance; it simply means that they were not found at autopsy. They could have been carried away or further split, although a perusal of Kutscher's work on Antipeptone convinces one that these bases are very resistant to digestive ferments at least.

Two crystalized fractions of monoamido-acids were secured. One fraction weighed 0.350 g. The melting point of the crystals was 173° ; melting was accompanied by dissociation. The specific rotation (in 20% HCl) was $\alpha_D = +15.6^{\circ}$ at $t = 15^{\circ}$. The elementary analysis gave the following result:

0.0982 substance:	0.1985 CO_2 and 0.0884 H_2O .
0.100 g. " :	0.0112 N.

Calculated for $\text{C}_6 \text{H}_{13} \text{NO}_2$.	Analysis.
C = 54.89%	55.12%
H = 10.00%	10.08%
N = 10.70%	11.2%

The crystals gave the test of Scherer. The substance was undoubtedly d-leucin.

The second fraction weighed 0.612 g. The specific rotation (in 10% nitric acid) was $\alpha_D = +24.3^{\circ}$ at $t = 15^{\circ}$. The elementary analysis gave the following result:

0.1854 substance:	0.2437 C_2O and 0.0913 H_2O .
0.1412 " :	0.0152 N.

Calculated for $\text{C}_4 \text{H}_7 \text{NO}_4$.	Analysis.
C = 36.06%	35.76%
H = 5.31%	5.51%
N = 10.54%	10.77%

The substance was asparaginic acid. For a further confirmation, the copper salt was prepared and analyzed. It contained 22.82% Cu; the formula requires 23.06% Cu. This is, to my knowledge, the first time asparaginic acid has been found in tissue.

Phenylalanin was carefully sought for, but with negative results.

The watery extract, studied with the methods of Kühne and Neumeister, contained deuterio-albumoses, but no true peptone.

The total fat in the etherial extract was 19.61 g. It was purified by solution in acetone, filtration and evaporation to dryness; solution in petroleum ether, filtration and evaporation; solution in ether, filtration and evaporation. The fat was of a pale brown color. On analysis it gave the following figures:

Free acid number	.	.	.	27.51
Saponification number	.	.	.	129.8
Ether number	.	.	.	107.29
Iodine number	.	.	.	46.4
Volatile acid number	.	.	.	3.14 (for 1 g. fat)
Acetyl number	.	.	.	38.1

In explanation of the chemical nature and values of fats, two now attested facts in the physiology of fats must be stated. When fats are ingested they are split, and in the process of absorption are reconstructed into their original states. Thus when all the depot-fat has been derived from ingested fat, that depot-fat follows the type of the ingested fat and is not specific to the host. The mutton-dog of Rosenfeld has become a classic illustration of this fact. When, on the other hand, an animal forms his fat from sugars, these fats are specific to the species. A dog and a sheep will form their different fats from the same sugar. The general laws may be thus stated: Depot-fat derived by synthesis from carbohydrates is specific to the species; depot-fat derived from ingested fat is specific to the diet. It is thus apparent that human fat will be found to vary within wide limits, since

much of our body fat is derived from ingested fat. I have for several years been engaged in analyses of human fats, the data, however, are not yet extensive enough for detailed publication. The values for the depot-fat are, however, fairly worked out.

In the following table are presented from personal analyses figures for normal human fat taken from the subcutaneous connective tissues :

Free acid number	1-3.5
Saponification number	175-185
Ether number	175-180
Volatile acid number	0.2-0.8
Iodine number	22-35
Acetyl number	2-8

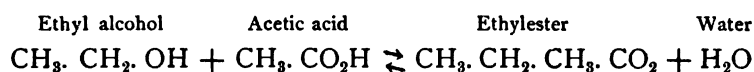
The variations in the figures express only the common variations.

For the fat of organs I am not yet able to present definite values. In a tentative way the results up to the present seem to indicate that the fat of the organs differs from the areolar fat in a higher proportion of non-fat, in a higher proportion of oxyacids and alcohols, in a higher free acidity, and often in a higher iodine number independently of the alcohols. I have as yet not sufficient analyses to enable me to state how constant or how pronounced are these differences; sometimes they are very slight. A comparison of the fat from a diseased organ with normal depot-fat is not a fair comparison, but it is the only one now possible.

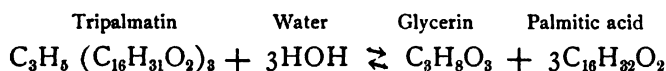
A comparison with the figures in the first table indicates several differences. The fat from the degenerated liver has a much lower saponification number; this indicates simply an excess of non-fat. The acid number is very high, ten times as high as normal; and while the volatile free acidity was also very high, it comprehended but a small part of the acid number, the rest of which must have been due to free fatty acids high in the series, in all probability to stearic, palmitic, and oleinic acids. This is a markedly pathological condi-

tion. The iodine number is somewhat above the normal, due doubtless to an excess of cholestrine. The acetyl number was very high, and this represents a heavy excess of oxyacids and alcohols. These must have been largely oxyacids, since had the increase consisted mainly of alcohols the iodine number would have been much higher. To recapitulate, the notable pathological alterations in this fat were the excess of free fatty acids and of oxyacids and alcohols.

A direct explanation of these abnormalities cannot be given. It is clear that the nature of changes in fat metabolism must be worked out along broad experimental lines. The recent studies of Cassel and Loewenhardt have shown that the reversibility of ferment action first described by Hill and Emmerling holds good for fat-splitting ferments. This phenomenon is in all probability simply a manifestation of the law of mass action. The attempt is made to establish a state of chemical equilibrium. A popular illustration of this law is furnished by the formation of esters.



The reaction is reversible and reciprocal, and when the mixture has come to rest in the chemical sense, all four substances are present and the system is in a state of equilibrium. The same thing must be true of the splitting of fat which are also esters. An example may be used with palmitin.



It is obvious that until the study of the fat metabolism is undertaken from this point of view, discussion of pathological states is vain.

The normal liver contains from twenty to sixty grams of free fat. There was therefore no increase in the quantity of fat in the degenerated liver. The normal liver may indeed contain one hundred grams of free fat. In the largest of the livers with fatty infiltration the fat may be run as high as

three to four or even five hundred grams. Cases of contracted cirrhosis of the liver usually contain from forty to eighty grams of free fat and may contain over one hundred grams. It is clear from these comparisons that it is entirely wrong to speak of the liver in acute yellow atrophy as being a fatty liver; there is no evidence, in this case at least, that the quantity of fat has been disturbed in the least. It is of course possible that an increased quantity of fat was present and that it was removed or burned, but of this there is no evidence. There is also no evidence that fat was formed in the degeneration of the protein substance of the liver cells. To judge from this one case, in acute yellow atrophy of the liver we have a necrosis, according to all analogy of bacterial or enzymic origin, not associated with the formation or accumulation of fat in the liver.

The combined fat, obtained after digestion of the extracted residue, according to the methods of the Pflueger school, was 1.005 grams. The normal liver contains from three to seven grams of combined fat. This reduction is approximately proportionate to the reduction in the mass of the organ.

SUMMARY.

Analysis of the liver from one case of acute yellow atrophy of the liver yields the following results and conclusions:

The loss in substance was disproportionate to the reduction in the total weight; *i.e.*, the liver was hydremic.

The nitrogen was proportionate to the dried residue.

The liver contained no glycogen.

The liver contained albumoses.

The quantity of ash was normal.

The liver contained no hexon bases.

The liver contained notable quantities of leucin and asparaginic acid, the products of hydrolysis of protein.

The quantity of fat was not altered from the normal.

The fat contained an excess of free fatty acid and of oxyacids and alcohols.

Acute yellow atrophy of the liver ought not to be classed as a fatty degeneration.

MULTIPLE ANEMIC INFARCTS OF THE LIVER.*

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† True anemic infarcts of the liver are rare. This fact, together with the possibility of clearing up some of their etiological factors, are the reasons why I report this case.

Before proceeding it will be well to define anemic infarcts, since formerly the term was applied to many conditions. In this article the term "infarct" will mean an area of necrosis due to the shutting off of the blood which supplied the part with its nutrition. To-day all concede that true anemic infarcts occur in the brain, heart, spleen, and kidneys because of their end-artery blood supply; but because of its two afferent blood streams, it was thought for a long time that anemic infarction of the liver was impossible. However, when it is remembered that the portal supply probably has nothing directly to do with the nutrition of the organ, that the hepatic artery distributes the blood to Glisson's capsule for nutritive purposes, that the hepatic vein returns this blood so used and that the arterial radicals are end-arteries, then it will be seen that a plugging of the hepatic artery or vein will cause an infarct. The literature of anemic infarction of the liver has been confusing because of the former free use of the term "infarct." Hence it will be well to take up this part of the subject somewhat in detail.

The first case reported was that of Ogle in the "Transactions of the Pathological Society of London," in 1895. The patient was a male who was admitted to the hospital because of a fractured spine. At autopsy Ogle found infarcts in the liver due to thrombosis of the hepatic artery, together with infarcts in the spleen and kidneys. He thought the thrombi arose from emboli which had loosened from a calcareous

* Read March 30, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, at Cleveland, Ohio.

† From Pathological Laboratory Medical Department, University of Michigan.

aortic valve. I have no doubt that this case was one of true infarction, even in the absence of microscopical examination, because of the presence of infarcts in the spleen and kidneys, the thrombus in the hepatic artery, and because of the calcareous aortic flap.

In the same volume Pitt reported a case which he called anemic infarct of the liver. His patient was a male admitted into the hospital in a state of unconsciousness and hemiplegia. He became better, but later died. At autopsy Pitt found a thrombus in the descending thoracic and abdominal aorta with complete obliteration of the lower branch of the splenic, the left renal and right mid-cerebral arteries, the right hepatic vein, and a softening thrombus in the portal vein. Sections presented sharply-defined pale areas which showed an "increase of cells in the portal canals and in the linear tracts radiating from them, producing a condition of early cirrhosis." The capillaries were engorged with blood in these limited areas. He did not mention any necrosis, and this alone would show that he did not have a case of true anemic infarct of the liver as we now know the term.

Osler ("Transactions of the Association of American Physicians" in 1887) reported a case diagnosed as "anemic infarct of liver." The patient was a sailor addicted to the use of alcohol. When he was admitted to the hospital he had dropsy, hematemesis, and ascites. At autopsy the liver was large, weighed 2,400 grams; the capsule was slightly thickened especially along the right border. The organ was extremely cirrhotic; the lobules were mapped out into small areas which did not project above the cut surface, but gave a characteristic granular appearance. In the right lobe he found areas, differing both from normal liver and from this cirrhotic liver. These areas were mottled, friable, and dry. Few strands of connective tissue passed through these areas, but the cirrhotic character was lost. Microscopical examination showed that these areas contained innumerable red blood cells infiltrating the entire tissue. The liver cells were small and fatty and in places indistinguishable. The portal vein presented a soft brown thrombus, which occupied the

upper part of the trunk, but did not completely obliterate the lumen. Branches which passed to the right lobe contained closely adherent light brown thrombi. The branch which passed to the anterior lateral region, which contained the infarct, contained a firm, solid, partly laminated clot, of recent origin, which completely obliterated the lumen. The facts that the liver cells were for the most part not necrotic and that the thrombus was in the branches of the portal vein, made his case probably not an anemic infarct, but a case of atrophy caused by the congestion and hemorrhage due to the thrombus in the branches of the portal vein.

Chiari ("Centralblatt für Allgemeine Pathologie und pathologische Anatomie," Vol. IX.) reports seventeen cases of "Atrophische rothe Infarct des Lebers." The infarcts of fifteen cases resulted from emboli in the portal branches and the other two from thrombus caused by carcinoma. In all cases the so-called infarct was wedge-shaped with the apex inward. Microscopically, he said that the liver cells were only atrophic and not necrotic. This, then, would prove that they were not infarcts in the accepted sense of the term, and he himself calls them Atrophic Red Infarcts. These may be local atrophy due to anemia.

Lazarus-Barlow ("British Medical Journal," 1899) reported another case. His case was a man who was crushed between the buffers of two railway coaches. When admitted to St. George Hospital he was in a state of collapse, but showed no abdominal symptoms. The only sign which could be found was an enlarged liver dullness on the right side but sharply limited toward the median line. Later vomiting began, but the vomitus was free from blood. Hiccough began at the same time and the man died. At autopsy the abomen was filled with fluid blood tinged with bile. All the organs were practically normal, except the liver. The right lobe of the liver showed a large rupture which extended from the anterior margin near the gall bladder over the convex surface to the posterior aspect. The rupture was irregular in outline, bifurcated over the convexity, and measured seven inches in length and half an inch in its greatest width. The

rupture was filled with a clot covered with bile-stained fibrin. Near the center of the rupture he found a pale yellow mass, ovoid in form, superficial and wedge-shaped in section. This mass was two by one and a half inches, and one inch in greatest depth. It looked like normal liver except for the absence of the reddish brown color. In the center of the mass, branches of the portal vein contained thrombi. This he called an anemic infarct, and added further that at the apex of the infarction and along its inferior margin was a large bile duct. Still deeper in the substance of the organ was a large blood clot irregularly disposed which reached very nearly to the inferior surface of the liver. In certain regions ("notably in the center of the liver and tracked into apparently normal liver and superficially on either side of the rupture") he found that the liver substance had a purplish hue and that it was raised above the cut surface. Other than these characteristics these regions were identical with the normal liver. He called these hemorrhagic infarcts because they assumed a wedge-shape. His conclusions presented two points against anemic infarct: First, he did not mention any microscopical examination, and if he had made no such examination he could not be sure of the necrosis; second, the thrombus was in the portal, and all previous cases and experiments proved that no necrosis results from plugging of the branches of the portal vein. Had there been any necrosis, the large rupture and the consequent hemorrhage might have explained it easily, the former by a tearing loose of the area and the second by a compression atrophy.

In 1899 Castaigne presented to the Société Anatomique de Paris the result of an autopsy. Patient had an aneurysm at the apex of the heart, with thrombosis of the heart, and embolism of the spleen, kidneys, and lungs. In the liver was a hemorrhagic infarct resulting from the obliteration of an intra-hepatic branch of the portal. He mentioned no necrosis; on the other hand the area was hemorrhagic, or at least greatly congested, so I would not include this under the term "infarct."

In 1900 Heile published a case in the "Beiträge zur pathologische Anatomie," which showed what he called a traumatic anemic necrotic infarct. His case was a male, aged thirty-five years, who fell from a bath-tub, striking his right side against a step. He became collapsed, and his pulse was not perceptible when he came to the hospital. He died, and at the autopsy the liver was normal except for a rupture fifteen centimeters long on the upper surface of the right lobe. The neighboring liver parenchyma was an "infarcted mass" depressed on section, wedge-shaped, and made up of necrosed tissue. The hepatic arteries and portal vein supplying this area contained thrombi. All the vessels at the liver hilus were normal. All the necrosis present might have arisen from the mechanical loosening of its nutritive vessels, and the same was probably true of the thrombi found, and consequently I would not call it an anemic infarct.

Longcope reported ("University of Pennsylvania Medical Bulletin" of August, 1901) two cases which he called Hepatic Infarcts. The first was a male who gave a history of dizziness, vomiting of blood, pain in the stomach, and loss of weight. Physical examination showed an increased liver and spleen dullness, and a distended abdomen. Tapping the abdomen gave a large amount of clear yellow-green fluid. At post mortem they found a primary adeno-carcinoma of the stomach with secondary nodules in the pancreas, retro-peritoneal structures, spleen and liver; thrombosis of the hepatic, splenic, and mesenteric veins, and infarction of the liver. Microscopically, the infarcts of the liver consisted of a large area of necrotic liver tissue which still retained its original form, but whose cells did not stain. The capillaries of the central zone of the lobule were markedly dilated and contained necrotic leucocytes, red blood cells, and desquamated endothelial cells. The hepatic vein at the base of this infarct contained a thrombus which in places occluded the lumen. Throughout the thrombus were many bacilli, clumped in masses and singly. This case, then, would be a true anemic infarct of the liver according to the definition given above.

Longcope added another case of liver infarct in the same issue of the "University of Pennsylvania Medical Bulletin." This case was one of carcinoma of the stomach with metastases in the liver accompanied by infarction of the liver. We need only consider the liver in this case. It contained many secondary deposits and on the ventral surface of the right lobe he found a slightly elevated, wedge-shaped, circumscribed nodule which measured about three inches in depth. Microscopically, this area contained a thrombus in one of the portal veins. The liver around this vessel retained its structure, but the cells stained poorly. The portal capillaries were dilated. The liver cells of the peripheral zone were somewhat degenerated, those of the central vein were distorted, some of them showed beginning fragmentation. This case, to my mind, was not anemic infarct because he mentioned no complete necrosis and the thrombus was in the portal vein. This resembles those of Chiari.

In this connection I might speak of two cases reported by Pitt at the same time as the one cited above under his name. Both were cases in which the lumen of the portal vein was occluded, one by a thrombus and the other by an "antemortem clot." Neither showed necrosis and he did not report them as instances of anemic infarction. These cases show that the portal circulation may be closed, and yet no necrosis occur.

With this summary of the clinical side, a study of the experimental work on hepatic infarction will be in order. In 1876 Cohnheim and Litten ligated the hepatic artery, and necrosis of the liver resulted. They ligated the portal vein, and an intense congestion of the lobules of the liver resulted, but no necrosis. Since then various observers have done much experimental work along these lines and have arrived at varying results.

In 1888 Rattone worked upon dogs. He ligated the hepatic artery and injected an emulsion of wax and cork into the portal vein. He found in each experiment what he called hemorrhagic infarcts. The explanation of the findings to my mind is that he had an anemic necrosis, caused by the

ligation of the hepatic artery, and to this was added the congestion caused by the plugging of the portal circulation.

In 1898 Doyan and Dufourt reported results similar to those of Cohnheim and Litten and explain the various results obtained by the others, by the fact that the hepatic artery with all its branches had not been tied. Dujarier and Castaigne took up this line of work, stimulated to it by the case which Castaigne presented to the Société Anatomique de Paris, and reviewed the literature of the work done up to that time. They found that Arthand and Butte, Stolnikoff and de Dominicis reported that dogs did not die in a short time after ligation of the hepatic artery, some of their animals having lived twenty days without any necrosis. Jansen gave varying results of similar work in the rabbit. Some of his animals lived longer than those of Cohnheim and Litten, and at post mortem cysts replaced the areas of necrosis. The conclusion of their own work was that necrosis could occur from ligation of the hepatic artery, but in some cases it was due to bacteria. They explain the various results obtained as due to the fact that the liver receives its blood supply from other sources than the hepatic artery, at least in the dog, and that these vessels must also be ligated in order to get complete necrosis of the liver.

To condense the literature, I would say that: First, the cases of Chiari, Pitt, Osler, Lazarus-Barlow, Heile, and the second case of Longcope were probably not true anemic infarcts. Second, that the case of Ogle and the first of Longcope were undoubtedly true anemic infarcts. Third, anemic necrosis has been produced experimentally by ligation of the hepatic artery, and ligation of the portal vein will not cause necrosis, but a marked congestion.

The case I will present was admitted to Dr. Dock's clinic of the University Hospital, February 18, 1899, with the following history: Mr. S., Cheboygan, Mich., American, aged 39; blacksmith. When admitted he complained of "swelling of the feet," "cough," and "loss of breath."

The family history was negative and the patient had had

the usual diseases of childhood, but had had no venereal disease.

In July, 1898, he began to have palpitation of the heart. Later he noticed that his limbs were swollen, and he began to cough.

Status Præsens: February 19, 1899. Patient looks sick, breathes with difficulty; sits up in bed; somewhat cachectic; mind clear; joints normal; no enlargement of glands; marked edema of legs which extends to hips, but is confined chiefly to the legs; eyelids puffy; scrotum and penis, somewhat edematous; height five feet nine inches. Frame large; musculature rather large, especially that of the arms, but soft; that of the legs large. Subcutaneous tissue over the parts thin but not edematous. Skin somewhat anemic with sallow tinge.

Thorax: large, symmetrical, epigastric angle of good size; breathing short, hard, and labored. **Percussion:** Right lower lung boundary is in sixth intercostal space in the nipple line, descending very little; eighth rib in axillary line; note has a tympanitic quality. Left lower lung border in nipple line; dullness is encountered over fourth rib, above which is a tympanitic sound. In axillary line, dull tympany down to eighth rib. **Auscultation** gives increased vesicular murmur over the upper chest; below the second rib, right side, gives numerous crepitant râles both on inspiration and expiration. On the lower left side the breathing is not so strong and râles are not present. Vocal fremitus is increased. **Heart:** Faint apex beat in sixth intercostal space in the anterior axillary line. **Percussion:** Dullness begins in the third intercostal space in the sternal line, extends to the left outside the nipple in the fifth intercostal space for one inch and to the right to one-half inch outside of the sternum. **Auscultation** over the apex gives a deep sonorous musical sound which resembles a murmur in systole, and at times in diastole. Breathing influences it somewhat, yet when the patient holds his breath it is present but not so marked. This murmur is heard over the entire precordia. Nearer the sternum a fairly loud diastolic murmur is present. It is loudest just to the left of the sternum in the third intercostal space to the second and also in the second intercostal space of the right side. In the axilla in diastole, a faint murmur is heard. Pulse is ninety-four, regular but small, weak, and quick. No capillary pulse noticed.

The abdomen is above the level of the ribs; liver extended one and a half inches below the level of the ribs; splenic dullness begins on eighth rib axillary line and extends nearly to the edge of the ribs; neither spleen nor liver palpable; dull, tympanitic note over the intestines. The blood count was negative. The sputum for twenty-four hours was about sixty cubic centimeters, viscid, frothy, slightly muco-purulent, contained much blood. Stain for tubercle bacilli and elastic tissue negative; staphylococci and streptococci and diplococci were present in the stained specimen.

The urine contains a small amount of albumin, few hyaline and granular casts, and small amount of pus. Urine drawn by catheter and inoculated into animals gives a growth of colon bacilli.

The diagnosis of the case was: Double aortic lesion, mitral regurgitation, relative tricuspid regurgitation, dilatation and hypertrophy of heart; general anasarca. The treatment was, medicinally, strophanthus, strychnine, and nitro-glycerine.

The condition of the patient gradually grew worse, his heart more feeble, his breathing more difficult, and the edema of leg more marked, 5,650 cc. of fluid having been withdrawn from the legs during twenty-four hours commencing February twenty-seventh. On April 6 8,000 cc. were withdrawn. On April 9 patient died, and came to autopsy April 10.

Autopsy protocol (condensed).

Died April 9, 1899. Autopsy by Dr. Warthin, April 10, 1899.

Body well built; no anomalies of skeleton; length, 168 cm. The skin is stretched, partly rough and shining, rough over lower extremities; marked edema of the lower extremities; moderate universal edema. In the skin of center of the abdomen are some small pigmented spots, pale in the center. There are a number of puncture marks over the lower extremities. Panniculus is small in amount. Musculature small and flabby. Patient well haired, with slight grizzled beard. Body heat is present at the time of autopsy. Moderate hypostasis over the dependent parts of the body and over the lower extremities, more marked on the right than on the left. Brownish slimy fluid exudes from the mouth and nose. On main section the amount of fat slight.

Head: Scalp and periosteum negative.

Brain: Moderately firm thrombus in the right middle cerebral artery; areas of softening extending over and involving left parietal lobe, anterior to the central fissure.

Thorax: Diaphragm on the right at the fifth rib, on the left at the fifth interspace. Muscles of the thorax are pale, brownish red, moist, but do not tear easily. First rib cartilage is ossified. Mammæ negative. Mediastinal tissue is very edematous, and lymph glands are enlarged. Apex of heart is at sixth interspace in the anterior axillary line. Heart extends to right sternal line. The pericardial sac is distended and contains about 400 cc. of golden-yellow turbid

fluid containing small flakes of fibrin. Heart is about four times the size of patient's right fist, and weighs 737 grams. The auricles and ventricles are enormously distended, especially upon the right side. Muscle fibers of the auricle are separated from one to two millimeters, the wall between the fibers resembling semi-translucent parchment. Heart measures 20 cm. long, 10 cm. thick, 13 cm. wide. There is a double apex separated by a shallow groove, apex of the left ventricle being the lower. Epicardium, especially over the auricle, shows small bits of fibrinous exudate, which are present also in small particles over the root of the aorta and pulmonary artery. Near the fresh exudate are a number of small tags of organized exudate. The pericardium throughout is dull, shiny, and thickened; sub-pericardial vessels are deeply congested; sub-pericardial fat is edematous, but not increased in amount. The heart contains a large amount of dark fluid blood, a few currant jelly clots, and many yellow clots having a fatty appearance. After emptying blood from the heart, the right auricle and ventricle collapse, the rounded left ventricle retains its form. Right heart: Auricle and ventricle enormously distended. Tricuspid orifice admits five fingers. A small thrombus is present in the tip of the auricle, otherwise it is negative. Right ventricle wall measures 4 mm. Auricle wall measures $\frac{1}{2}$ to 1 mm.; tricuspid flaps negative. The pulmonary semi-lunar flaps appear larger than normal, and the opening dilated. The trabeculæ of both auricle and ventricle are flattened. Left heart: The left ventricle wall measures 12 to 15 mm., and the auricle is not nearly so distended as the right, but shows a separation of its muscle fibers. Near the ear there is a firmly attached organized thrombus. The mitral orifice admits four fingers, and the flaps are thickened. Aortic flaps are thickened, retracted, admit finger, and are inadequate. Heart muscle is brownish red, and there are a few fibroid areas in the ventricle. The columnæ carneæ are moderately increased in size.

Lung: The left pleural cavity contains 500 cc. pale yellowish fluid. No adhesions except posteriorly, where

they are easily separated, and a few anteriorly, to the pericardium. Lung is partially collapsed, its pleura dull, cloudy, and covered everywhere with fibrinous exudate, most marked over the lower border and over the lower portion where the exudate is partially organized. Color of the lung is gray red, with moderate anthracosis. There are many firm airless areas along the lower lobe and the anterior edge of the upper lobe; these areas project above the surface of the lung. Cut surface is brownish red in color, moderately rich in blood, and yields on pressure an abundant, slightly turbid, frothy fluid. The airless areas are dark brown red in color, and project above the surface. Bronchi contain slimy fluid, and the mucosa is injected. Smaller branches of the pulmonary contain fresh and partly-organized thrombi. The most marked changes are edema, infarction, induration, and thrombosis.

Right lung: Pleural cavity contains about one-half liter of creamy pus. Many firm adhesions to the anterior surface of lung which extend for a short distance only, and over the base where the lung is firmly attached to the diaphragm. Lung is almost entirely collapsed, and surface covered with a thick fibrino-purulent exudate so adherent that it is necessary to remove the lung with the diaphragm. On the lower border is an old hemorrhagic infarct over the base, brownish in color. Thick, irregular stringy masses of yellow fibrin, firmly adherent to the pleura, covered the surface. Lower and middle lobes contain no air. Cut surface is moderately rich in blood, brownish red in upper lobe, gray in color elsewhere, and yields abundant foamy exudate on pressure. Mucosa of bronchi is injected and covered with yellowish mucus. The bronchial glands and thoracic duct are negative.

Abdomen: Liver and intestines project out of abdomen on making chief incision. Cavity is filled with golden-yellow, very slightly turbid fluid, about a liter and a half in amount. The lower edge of the stomach is two fingers' breadth below the ribs in the right nipple line. The omen-

tum is poor in fat, brownish yellow in color and edematous; vessels congested.

Spleen: Very irregular in shape, has a deep furrow in the anterior surface and capsule over furrow is thickened and opaque. Capsule is thickened throughout and contains many small white adhesions. Cut surface presents two large yellow areas extending through the spleen and corresponding to the areas of deepest furrows. These areas are slightly elevated above the cut surface. Consistency of the spleen is increased, firm, and follicles not evident. Color is gray red. The main artery contains a partly organized thrombus, three-quarters of the lumen being shut off by it.

Adrenals: Fairly well preserved.

Left kidney: Fatty capsule is poor in fat and fibrous capsule is slightly thickened but not adherent. The surface of the kidney is smooth, a gray-brown-red in color; *venæ stellatæ* not congested. Cortex slightly increased in thickness. Glomeruli easily seen as red points. Color of parenchyma is gray-brown-red. Pelvis is negative.

Right kidney: Cortex slightly more granular and contains a few furrows; otherwise same as the left.

Ureters are negative.

Duodenum: Contains grayish slimy substance. Just below pylorus are four ulcers, the largest about 4 cm. in diameter. Its edges are black, and the floor is covered by a black, coffee-ground-like substance. This ulcer extends through the mucosa and has a firm nodular mass size of chestnut behind it. Many branches of the superior mesenteric contain firm thrombi.

Stomach shows beginning post mortem digestion; mucosa thin, pigmented, and has small areas of hypostatic congestion and one hemorrhagic area. Two ulcers similar to those found in the duodenum are present in the fundus of the stomach which are about the same size but not so deep, one being partly healed and has a thickened base.

Liver: It is oblong in shape; diameter of the right lobe increased antero-posteriorly and diminished in other directions. Underneath the capsule the surface shows twenty to

thirty firm, slightly elevated yellowish spots, surrounded by reddish zones. Surface of the liver is granular; has a fatty shine, and the veins are enormously dilated throughout.

On section these wedge-shaped areas are firm and cheesy with the base of the wedge outward and apex inward, involving only four to five lobules as a rule.

Gall bladder: This contains dark brown fluid and the walls are extremely edematous.

Portal vein is negative.

Pancreas is negative.

Testes are edematous.

Material was taken from all the organs, fixed either in mercuric bichlorid, Müller's fluid, formalin or alcohol, embedded in paraffin, sectioned and stained with hematoxylin and eosin, or Van Gieson's mixture.

Microscopic examination: Lung: Sections from different parts of both organs show a general chronic congestion, many scattered thrombi and hemorrhagic infarcts, both fresh and old; scattered areas of broncho-pneumonia. The pleura shows a sub-acute fibrino-purulent pleuritis. Heart: Sections show a great hypertrophy of many cells, and brown atrophy of others. The pericardium shows a fresh fibrinous pericarditis. The spleen shows many healed anemic infarcts and a condition of general chronic congestion. Kidneys show moderate atrophy and chronic congestion with many healed anemic infarcts. Mesentery: The firm masses which filled some of the vessels are fresh thrombi. The intestinal tract shows a chronic gastro-enteritis. The ulcers of the duodenum and stomach are anemic. Bone marrow shows lymphoid hyperplasia.

Liver: The liver as a whole shows marked atrophy, chronic congestion, some fatty infiltration, and small amount of fatty degeneration. Sections from the small light-colored wedge-shaped areas show that the liver tissue is necrotic. The form of the liver rods is preserved as well as the outlines of the connective tissue, with the vessels and bile ducts in Glisson's capsule, but the nuclei of all the tissue cells of this area fail to stain. The branches of the hepatic artery

contained in these islands of the capsule contain old thrombi made up of the granular debris of red and white cells and fibrin, but they show no organization. Around the periphery of each mass, fibroblasts are beginning to grow into the area and the bile ducts show signs of proliferation. In some places the remains of a hemorrhagic zone show as red blood cells which are degenerating and losing their hemoglobin, and phagocytes which contained hemosiderin and hematoidin. In sections stained by the Weigert's fibrin stain, the granular and stringy remains of the cells in the liver lobules of the peripheral portions of these wedge-shaped areas retain the stain as much as the fibrin in the thrombus. The central portions of these areas do not react in this manner to this stain. Other sections were stained with Polychrome Methylene Blue. These sections show scattered plasma cells around the periphery of the area, more especially directly under the capsule, where they are twice or three times as numerous as the liver-cell side of the mass. No mast cells are present in any portion of the sections. In no sections were germs present. These areas showed a condition diagnosed as multiple sub-acute anemic infarcts.

All other organs were negative.

Pathological Diagnosis: Aortic Stenosis and Insufficiency; Relative Mitral Insufficiency; Extreme Dilatation of Heart; Thrombosis of Right Auricle; Thrombosis of Vessels with Infarction of the Brain, Lungs, Spleen, Kidneys, Stomach, Duodenum; and Liver; Broncho-pneumonia; Chronic Congestion of all organs; Fresh Fibrinous Pericarditis; Sub-acute Purofibrinous Pleuritis; Chronic Gastro-enteritis; General Edema; Secondary Anemia.

That this case presents true anemic infarcts there can be no doubt, for the following reasons: (1) Investigators upon whose work and technique we can rely have produced anemic necrosis of the liver by ligating the hepatic artery. (2) This case presented undoubted infarcts with the various sequelæ in the brain, heart, lungs, spleen, and kidneys. (3) The conditions for infarction were most favorable. The heart was very weak, and consequently the blood flow was slow.

The liver was in a condition of chronic congestion and this made the flow much slower, so that an embolus, once lodged, easily shut off the blood supply or a primary thrombus could easily have started from the lesion produced by the poor nutrition. (4) The appearance of the areas was most certainly that of an infarct. The outlines of all structures were preserved, but no nuclei would stain, the branches of the hepatic artery supplying these areas with blood were completely blocked by thrombi, the leucocytes of which had lost their chromatin and the red cells their hemoglobin. Surrounding the whole area was a zone of old red blood cells and a beginning organization.

To state my conclusions briefly: (1) This was a case of multiple anemic infarction of the liver, accompanying similar conditions found in the other organs. (2) That anemic infarcts occur but rarely and then only when the liver labors under such disadvantages that it cannot overcome the results of an embolism or thrombosis in its congested hepatic vessels.

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ON THE ERYTHROGENIC SPLEEN OF MEPHITIS MEPHITICA.*

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It has been my good fortune to obtain histological material from six specimens of *Mephitis mephitica*. The spleen of this animal presents a condition which, so far as I am aware, has hitherto escaped notice. As this condition has some bearing upon a general problem of blood formation it seems worth recording.

In speaking of the spleen as a hæmatopoietic organ, Ehrlich and Lazarus¹ note the fact that in the lower vertebrates it is a locus of red corpuscle formation, whereas among the mammals only two species show evidence of such a function in their spleens during adult life.

Nucleated erythrocytes occur in the spleen of the mouse in considerable numbers, in the spleen of the rabbit in very small numbers.

Ehrlich and Lazarus also note that in the dog nucleated red cells are present only after bleeding experiments and are not found normally. The same can be said of the cat². In human spleens nucleated red cells occur only in leukæmic disease³.

Dominici³ has shown that in the rabbit the spleen can undergo a myeloid transformation. This change in the spleen he brings about by repeated bleeding and by the injection of typhoid cultures, and he also notes an indication of it during pregnancy. The myeloid transformation consists in a change in the cellular composition of the spleen pulp such that it simulates bone marrow.

In *Mephitis mephitica* the spleen shows evidence of active red corpuscle formation. The spleen of this animal is much larger in proportion to the body weight than in any other

* The above is published with approval of the committee as part of the work done under a Bullard Fellowship.

animal that I have examined. The amount of marrow in the long bones is comparatively small.

Histologically the bone marrow shows active formation of granular leucocytes; the finely granular cells, the cells with larger eosinophilic granules, and the mast cells. The most striking thing about the sections of marrow is their comparative paucity in cells of the erythrocyte series. Erythroblasts in all stages are present; but they do not form a prominent feature of the section as they do, for instance, in the normal marrow of the rabbit.

In the pulp of the spleen all stages of red corpuscle formation are present. The erythroblasts lie in the sinuses in great numbers and form a prominent feature of the section.

The fact that the spleen of *M. mephitica* functions as an organ for red corpuscle formation is of interest on account of its bearing upon the problem of the loci of hæmatopoiesis. In this animal the spleen normally divides with the bone marrow the labor of erythrocyte production. In the majority of the higher mammals the spleen is more nearly related to the lymphatic system than to the bone marrow. In *M. mephitica* the presence of erythrogenic tissue, which is found only in the bone marrow of most of the mammalia, emphasizes the serial character of the three hæmatopoietic organs.

The spleen of *M. mephitica* fits in very well with Dominici's conception of the spleen of the rabbit as composed of lymphatic tissue represented by the Malpighian bodies, surrounded by blood sinuses in which there is latent a myeloid tissue, masked by the normal prominence of red blood corpuscles and macrophages. In *M. mephitica* this myeloid tissue, as normally represented by erythroblastic tissue, is not latent but on the contrary very prominent.

It seems probable that a further systematic study of the hæmatopoietic organs of the mammalia would bring to the light other examples of a like nature. The spleen of *M. mephitica* normally performs one of the functions ordinarily resident in the bone marrow of other species and, if Dominici's conception is a true one, it is probable that animals

will be found in which the leucogenic function of the marrow is likewise shared by the spleen.

CONCLUSION.

The spleen of *M. mephitica* shares with the bone marrow of that animal the function of erythrocyte formation.

(NOTE. I wish to express my indebtedness to Dr. H. N. Kingsford of Dartmouth Medical School, Dr. E. E. Tyzzer, of Harvard Medical School, and to Mr. John Hartwell for their kindness in supplying me with material. I wish also to express my thanks to Dr. W. T. Councilman for his assistance and encouragement in this work.)

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EXPLANATION OF PLATE XXI.

1. Low power, showing erythroblasts in pulp.
2. Pulp sinus near capsule, showing group of erythroblasts. x 900.
2 mm. Zeiss Apochromatic.

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ON AMPHOPHILE LEUCOCYTOGENESIS IN THE RABBIT.*

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AND

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Contents: Introduction; I., Method of Research; II., Leucocytes of the Circulation; III., Structure of the Bone Marrow; IV., Cytology of the Bone Marrow; V., Amphophile Series in the Bone Marrow and the Circulation; VI., Histology of Mesentery and Omentum; VII., Cytology of the Mesentery and Omentum; VIII., Detail of Experiments; IX., Summary of Experiments; X., Discussion; XI., Conclusions.

The following research was undertaken to study the changes in the bone marrow following experimentally induced variations in the number of the circulating amphophile leucocytes.

From the results obtained we wish to advance additional evidence that the bone marrow is a locus of formation of the amphophile leucocyte and to formulate a hypothesis to explain how the supply of these cells is maintained.

According to the general law first laid down by Ehrlich the granular leucocytes arise in the bone marrow, whereas the non-granular leucocytes arise in the lymphatic system.

This law must be modified to some extent, as Ehrlich himself admits,¹ since Dominici² has shown that under certain experimental conditions the amphophile leucocytes of the rabbit, which belong to the class of granular leucocytes, may arise in the spleen. By repeated hemorrhage and by intra-peritoneal injection of typhoid cultures Dominici was able to

* Received for publication, Nov. 11, 1902.

produce a change in the spleen of the rabbit which he calls myeloid transformation. This change consists in the appearance, in the pulp of the spleen, of cells ordinarily found in the bone marrow. The cells with amphophile granules form a prominent feature of this picture.

In the present instance we need not be concerned with this locus of origin of the amphophiles, for, as will be seen, we have to do rather with the movements of ready-formed amphophiles than with cells formed during the experiment. The experimental procedures adopted do not lead to the "myeloid transformation" of the spleen which Dominici has demonstrated.

I. METHOD OF RESEARCH.

The general plan of the experiments was to cause the sudden withdrawal of a large number of amphophile leucocytes from the circulation and to observe how the bone marrow reacted to such a loss.

Various investigators have shown that if bacteria, their toxins, or various other substances producing leucocytosis, be injected into an animal the bone marrow exhibits a hyperplasia of the cellular elements with a partial or complete disappearance of the fat. All these experiments were of some days' duration and have demonstrated that, coincident with an increase in the number of the circulating leucocytes, there is a change in the cell composition of the bone marrow which can be interpreted as an exaggeration of its leucocytogenic function. Muir³ very properly compares this "leucoblastic" marrow with the "erythroblastic" marrow which follows repeated hemorrhages. Roger and Josué⁴ recognize similar marrow reactions. In the post-hemorrhagic marrow the erythrocytogenic function is exaggerated as shown by the predominance of erythroblasts. Rubinstein⁵ injected various leucocytotics into rabbits and studied the changes in the cellular composition of the blood and bone marrow. He used turpentine, extract of spleen, streptococcus toxine, and deutoalbumose to bring about the leucocytosis. Marrow was obtained by resection of bits of rib before

and after injection. Rubinstein found a decrease in the number of amphophiles in the marrow coincident with the increase of these cells in the circulation. He also found an increase in the large non-granular marrow cells. He concludes that leucocytosis is the exclusive function of the bone marrow. His views of leucocytogenesis are opposed to the genetic theory of Uskoff. We will have occasion to speak of Rubinstein's work later.

Ribbert⁶ is credited by Muir with the first experimental correlation of changes in the number of circulating leucocytes with changes in the bone marrow. Ribbert showed that the injection of pathogenic bacteria into rabbits was followed, in the bone marrow, by a disappearance of fat cells, their place being taken by marrow cells. His view of leucocytogenesis is substantially that of Ehrlich; viz.: "*Für die Bildung der Lymphocyten reichen die lymphatischen Apparate, für die der Leukocyten das Knochenmark völlig aus.*" (Virch. Arch., Bd. CL., p. 407.)

Roger and Josué have shown the reaction of the bone marrow to various injections. Similarly hyperpasia of the marrow has been noted by Milroy and Malcolm,⁷ Stockman and Grieg,⁸ Haushalter and Spillmann,⁹ Taylor,¹⁰ and others.

Muir's most recent contribution on the relation of the bone marrow to leucocytosis and leucocyte production gives a very complete picture of the reaction of the marrow to bacterial injections. He finds two sorts of change in the marrow, namely, formative and degenerative. The former is evidenced by decrease of fat and increase of amphophile myelocytes with many mitoses in them. The blood channels become sharply marked off. The number of polymorphonuclears is variable. The degenerative changes are shown in the giant cells, in the myelocytes, and in the polymorphonuclears. He concludes that chemotaxis is the important factor in bringing about the leucocytosis. The increase in the myelocytes in the marrow is due either to direct proliferation caused by the toxine or to a compensatory hyperplasia and over-regeneration to replace the lost polymorphonuclears.

In the experimental part of this research we are not con-

cerned with these gross hyperplastic reactions of the marrow that follow prolonged leucocytosis, but rather with the reaction of the marrow to simple withdrawal of amphophile leucocytes from the circulation. In order to attain the desired result the simple expedient of bleeding was not available on account of the complications introduced by the coincident erythroblastic reaction of the marrow which would mask the phenomena which we wished to study.

For that reason and also in order to have the locus of amphophile withdrawal available for histological study we chose to produce a transient aseptic peritonitis by the intra-peritoneal injection of relatively mild irritants. The materials used were either a very dilute suspension of turpentine in water (1-2000) or sterile salt solution (0.8 per cent) at 75 degrees Centigrade.

The intra-peritoneal injection of a small amount of either of these substances (5 cc.) causes a transient peritonitis which is accompanied by a considerable exudation of amphophile leucocytes into the mesentery and omentum and also into the peritoneal fluid.

By sacrificing the animal at various periods after the injection the composition of the exudation and the condition of the bone marrow could be studied. Leucocyte counts and the differential counts of stained smears from the blood of the ear vein, taken at intervals during the course of the experiment, yielded data as to the changes in the cell composition of the peripheral blood.

By sterilization of the material injected, controlled by cultural examinations of the peritoneal cavity on killing the animal, the absence of bacteria could be insured.

The experiments therefore yield data upon the behavior of the cell in which we are interested in three situations: viz., at the locus of its experimental withdrawal from the circulation (mesentery and omentum), at the locus of its normal habitat (the circulating blood), and at its supposed locus of origin (the bone marrow).

Before entering upon the account of the experiments it seems best to consider in some detail the normal histology and cytology of the three regions to be studied.

II. LEUCOCYTES OF THE CIRCULATION.

In the circulating blood of the rabbit we ¹¹ distinguish the following types of colorless nucleated cells:

1. Lymphocytes. Nucleus circular, chromatin often in masses murally arranged. Protoplasm non-granular and strongly basophilic.

2. Large mononuclears. Nucleus round, oval, or elongated and curved. Protoplasm non-granular, faintly basophilic.

3. Amphophiles. Nucleus polymorphous, chromatin frequently in masses murally arranged. Protoplasm granular; granules small, ovoid, oxyphilic, may have selective affinity for acid dyes under certain circumstances.

4. Eosinophiles. Nucleus polymorphous, chromatin in masses murally arranged. Protoplasm granular; granules large, ovoid, oxyphilic.

5. Mast cells. Nucleus polymorphous, poor in chromatin. Protoplasm granular; granules small, spherical, basophilic, metachromatic.

When enumerating the leucocyte types of the blood the question of the inter-relationships of the leucocyte forms must always arise. In the adult animal the question remains unsettled as to whether the cells which we recognize as types in the circulating blood are stages in the metamorphosis of a single cell or whether each is derived through a metamorphosis from separate cell ancestors.

We must admit that the highly differentiated leucocytes have all ultimately been derived from the mesenchymal cell. This differentiation must have gone on either within or without the blood stream or partly in both. Further it is conceivable that the mesenchymal cell which is to become a leucocyte may, during adult life, reach a certain degree of differentiation, and from there, by various sorts of metamorphosis, give rise to different leucocytes.

The tracing of the inter-relationship of the leucocytes, from the morphological point of view, therefore depends upon two elements, namely, the demonstration of enough intermediate forms to make a series and the determination of the locus of the differentiation.

We will now consider the evidence only as to the intra-vascular relation of the amphophiles of the rabbit to the other leucocyte forms. The question of extra-vascular relationship and of cytogenesis will be discussed in a later section.

The main question at issue is between the view that the amphophiles arise from a marrow cell on the one hand, and that they are a developmental form of a cell from the lymph nodes on the other.

In general terms the first of these alternatives expresses the view of Ehrlich, the second that of Uskoff. Dominici¹² has recently advanced a third and intermediate hypothesis, as follows: He recognizes two sorts of amphophiles in the circulation. One sort of cell is derived from a marrow cell and enters the blood fully formed. This agrees with Ehrlich's theory of leucocytogenesis. Dominici also recognizes a second source of amphophiles by development from a cell of the lymph node. Dominici, therefore, takes a middle ground. As to the first sort of amphophile, viz., the one which arises from a marrow cell and enters the blood stream in an adult condition there need be no dispute. Very many investigators have agreed to this view; that is, that the blood stream is not the locus of metamorphosis of the cells genetically related to the marrow cells. As to the second class of amphophiles, which Dominici describes, there may be some ground for discussion. We do not understand him to state that such cells are normally produced, but that under experimental conditions the lymphatic system may become a locus of origin of circulating amphophiles. He cites two ways in which this may come about:

First. The lymph nodes may become a locus of origin and of metamorphosis for amphophiles so that the cell passes through a myelocyte stage in the lymph node and enters the blood stream fully formed, just as they do from the marrow.

Second. Mononuclear cells may pass out from the lymph nodes and undergo a nuclear and protoplasmic metamorphosis in the blood stream in such wise as to become amphophiles. While we are far from wishing to dispute this state-

ment, we do feel that, in the normal rabbit and under the experimental conditions with which we are here concerned, this possible source of the circulating amphophile does not, if unconsidered, introduce an error.

To us the most striking thing about the blood preparations of the rabbit is the sharp differentiation between granular and non-granular cells. Almost equally striking is the line of demarcation between mononuclear and polymorphonuclear cells.

In smears that are so stained as to bring out all the different forms of granules and also the structures and outlines of the nuclei, we have uniformly failed to find evidence of a gradual acquisition of granules by an amphophile leucocyte coupled with a nuclear metamorphosis.

In short, we lean to the view that in the normal rabbit —

1. The amphophile leucocyte, as seen in the circulating blood, is a definite type of cell which, during its intra-vascular life, shows no variations in form which would permit of its being confused with any of the other leucocyte types.

2. That this cell normally enters the blood stream fully formed.

We will have more to say on this general subject of leucocyte relationships when speaking of the amphophile series of the bone marrow.

Statistically the amphophile leucocytes form about a half of the colorless cells of the circulating blood. We¹² have considered the following figures as representing approximately the range of variation in the percentage counts of the leucocytes in a normal rabbit.

Lymphocytes	45-55 per cent.
Large mononuclears . .	2-8 "
Amphophiles	40-50 "
Eosinophiles	0.5-1 "
Mast cells	4-8 "

In relation to volume the leucocytes of the rabbit vary greatly in number. In rabbits that seem normal in every respect the leucocytes per cubic millimeter may vary between

four and twelve thousand. Consequently the lymphocytes and the amphophiles, per unit of volume in the circulating blood, may fall anywhere within two to six thousand, and sixteen hundred to four thousand eight hundred respectively. On account of these wide variations in the number of cells per unit of volume we have found it convenient to compare different observations in the same animal or sets of observations in different animals by comparing the percentage differences.*

III. STRUCTURE OF THE BONE MARROW.

Considered as an organ the bone marrow can be described as an appendage to the general vascular system, situated in the cavities of the bones, in which a special capillary circulation comes in intimate relation with masses of free cells of the type of the formed elements of the blood.

In the diaphysis of the long bones the marrow presents as a cylinder bounded externally by the cortical bone. Between the marrow tissue and the bone is a delicate layer of connective tissue, the endosteum.

In the long bones a large artery enters through a foramen in the cortical bone, a vein emerging at the same place. At many points small capillaries pass obliquely through the endosteum from the marrow to enter the minute canals of the bone.

The bulk of the marrow is composed of fat and haemic cells in variable proportion, and is traversed by a vascular network. In the femur the arrangement of the vessels is as follows:

The afferent artery enters the marrow through an oblique foramen in the cortical bone which is situated on the post mesial surface. The axis of the foramen is directed obliquely downward and inward. When the artery emerges from the bony canal it passes through the marrow substance to about its longitudinal axis and there divides into two main branches

* The percentage difference is a decimal prefixed with a plus or a minus sign. The decimal is that of a fraction whose numerator is the difference and whose denominator is the first of the two observations to be compared. The plus indicates that the change is an increase, the minus that it is a decrease. See this Journal, Vol. vii., p. 194.

which are directed towards either extremity of the shaft. Each of these branches divides repeatedly. As the branches become smaller they tend to take a peripheral position as seen in cross section; that is, the smaller branches incline towards the peripheral portion of the marrow. The arteries break up into capillaries which tend in general to take a perpendicular course from the periphery towards the longitudinal axis of the marrow. Some of the capillaries, however, pierce the endosteum and enter the cortical bone. The capillaries which pass towards the center of the marrow finally empty into large, thin-walled venous sinuses which often partially surround the larger arteries and pass out of the marrow by the path of entrance of the artery. During the passage of the capillaries from the periphery of the marrow towards the central sinuses they pass through masses of cells which lie in spaces between the fat cells. These cell-filled spaces contain a delicate reticulum in which the cells are enmeshed. In places, the blood stream is not confined within endothelial walls, but wanders through channels in the reticulum and the masses of cells. The supportive tissue of the marrow consists in a small amount of connective tissue which forms the delicate endosteum, a small amount in the walls of the larger vessels, and the delicate reticulum of the sinuses.

A small amount of elastic tissue is present in the walls of the arteries.

Medullated and non-medullated nerves are described in the marrow. Lymphatics are not present.⁴

This description agrees substantially with that of Muir and of Roger and Josué.

IV. CYTOLOGY OF THE BONE MARROW.

The cells of the bone marrow, aside from the fat cells and those which enter into the formation of the vessels and supportive structure, can be divided into four groups, viz.:

1. Undifferentiated cells.
2. Cells of the erythroblast series.
3. Cells of the leucoblast series.
4. Giant cells.

To the first class belong certain cells which cannot be distinguished from certain cells of lymph nodes. These cells may be regarded as undifferentiated cells of mesenchymal origin. They are of small size, possess a round vesicular nucleus and a moderate amount of protoplasm which contains a basophilic cyto-reticulum. These cells are the same as those called myeloblasts by Nægeli.¹² Rubenstein calls these cells lymphoid cells, and says that though they have the same structure they stand in no developmental relation to the cells of the lymph nodes. Jolly¹³ regards these cells as stem cells from which by divergent lines of metamorphosis the erythroblast and leucoblast series of the marrow are produced. To us there appears every intermediate form between these cells and the least differentiated cells which can be certainly said to belong to the amphophile series. We need not concern ourselves with the second type of marrow cell, the erythroblast, as it does not figure in this research. The cells of this series are so characteristic in appearance as not to be confused with those which we are considering.

The giant cells or myeloplaxies need not to be considered in detail for similar reasons.

Taking up the cells of the leucoblast series, we find them divisible into three sub-classes on the basis of cell granulation, viz.:

Mast cells.

Eosinophiles.

Amphophiles.

With the first of these we have nothing to do in this article. It need only be mentioned in passing that they are characterized by the possession of basic protoplasmic granules.

The eosinophiles are a cell series whose end product is the eosinophile of the circulating blood. Rubinstein considers that they have the same ancestral cell as the amphophile.

Turning again to the amphophile series, we will try to trace out in some detail the relations of the circulating amphophile leucocyte to the cells of the bone marrow.

V. THE AMPHOPHILE SERIES IN THE BONE MARROW AND THE CIRCULATION.

As has just been said, the cells of the bone marrow can be put into groups and certain of these groups can be divided into one or more series. In so arranging the cells various obvious structural differences serve as criteria of differentiation. The main groups of marrow cells are separated one from the other by wide differences. At a first glance, for example, the erythroblasts appear distinct from the giant cells. When the remaining cells are examined more critically the series of cells that can be arranged within the leucocyte group begin to be apparent. These series seem to form in the mind from the most highly differentiated types that they contain. The polymorphonuclear cells with eosinophile granules separate themselves from those with amphophile granules. As we trace these cells backward we seek in succession cells which differ less from our chosen type than from the other cells of the marrow. Having found such a cell we proceed again from it as a base.

In following out this line of study one becomes convinced that a cell is present in the marrow that is so undifferentiated that it cannot be definitely said to belong to any one more than to any other cell series.

In this research we have to do only with the amphophile leucocyte. We wish to make clear our view of the genesis of this cell, as our interpretation of the experimental work to be detailed later depends upon the answer to this fundamental morphological problem. The finality and the originality of the view is problematical, but it must be advanced as a working hypothesis before the movements of the leucocytes in the living animal can be understood.

As has been said above, we recognize a cell in the circulating blood which is characterized by the possession of slightly oval protoplasmic granules and a polymorphous nucleus in which the chromatin tends to arrange itself in masses against the nuclear membrane. In a previous paper we have spoken of this cell in some detail. It has long been recognized that

identical cells are present in the bone marrow often in large numbers. A cell which is present in the active marrow presents the same features as the amphophile of the circulation, with the exception that its nucleus is more elongated, more tortuous, and contains less nuclear sap. The nucleus of this cell presents as a twisted ribbon which shows signs of segmentation. The protoplasmic granules are the same as the cell in the blood. From this cell with a strap-shaped nucleus a series of types can be found which lead by insensible gradations through a horseshoe nucleus to a cell with a large vesicular nucleus and numerous amphophile granules.

The next cell of the series is identical with the last, but is larger. From this point the series approaches the type of the undifferentiated cell through a stage in which the granules are present in small numbers in a group at one side of the cell. In these cells the cyto-reticulum becomes prominent and strongly basic. The series is interrupted by mitoses at various points before the development of the strap nucleus.

To recapitulate, we take as the earliest cell of the series the undifferentiated cell of the marrow which approaches most nearly to the type of the germ cell. This cell possesses a vesicular nucleus and a relatively small amount of protoplasm containing a basic reticulum. This cell first grows larger and acquires amphophile granules, which are at first irregular in size and are spherical, later ovoid, and so numerous as to fill the cell. Coincident with this development of granules the protoplasmic reticulum loses its basic property. The cell then grows smaller, the nucleus becomes first horseshoe-shaped, then strap-shaped and much elongated. With this change in the shape of the nucleus goes along a condensation in the chromatin.

The question of mitosis in these narrow cells has received much attention from Jolly¹³ in a recent article. He finds typical mitosis in marrow cells in a number of animals. The supply of myelocytes is undoubtedly dependent in part upon this. That is, up to the stage where the marrow cells acquire a strap nucleus they may undergo mitosis, the daughter being like the parent.

The circulating amphophile is then to be regarded as a highly differentiated mesenchymal cell. Structurally the differentiation is evidenced by the departure from the vesicular type of nucleus and by the presence of protoplasmic granules having certain stain-fixative reactions. We have discussed this point in a previous paper, but will refer again to certain reactions of these granules as seen in fixed tissue. A tissue containing an exudate of amphophile leucocytes fixed with Zenker's fluid and stained with Unna's alkaline methylene blue and eosin shows the granules of this leucocyte very distinctly. The same tissue stained with hematoxylin and eosin barely permits the granules to be seen. Tissue smears from the same areas show polymorphonuclear cells with typical amphophile granules.

The amphophile can be regarded as functionally highly specialized, as shown by its chemotactic sensibility and its relation to bacteria. Further it has, we believe, lost its power to divide. In this respect it parallels the specialization of the nerve cells.

VI. HISTOLOGY OF MESENTERY AND OMENTUM.

Mesentery. — For the purpose of this research the mesentery can be regarded as a delicate membrane passing between the body wall and the intestines. This membrane is invested on both sides by peritoneum which takes the form of a single layer of flat cells, and between these two layers are arteries, veins, nerves, lymphatics, fat, connective tissue, and elastic fibers. We are particularly concerned with this mid-layer of the mesentery. For the study of the peritonitis which we induce, the fat free portions of the mesentery are selected. These fat free spaces show the following arrangement of the structures in the mid-layer.

Vessels and nerves. — The spaces are traversed by what can be conveniently designated as vascular axes. These consist in an artery, vein, nerve, and lymphatic. These structures lie side by side and at certain points branch in such wise that the fat free space contains a relatively large meshed network of these axes. The blood vessels are col-

lateral to the larger vessels which pass directly to the gut through the fat trabeculæ. That is, the artery arises from a larger vessel in the fat trabecula and enters the fat free portion of the mesentery, but does not there break up into a terminal capillary network; on the contrary it traverses the space to enter another group of vessels in the same or another fat trabecula. The veins have a similar arrangement. The lymphatics are provided with valves at frequent intervals and are not through trunks like the blood vessels. A lymphatic can frequently be traced back until it is lost in the inter-fibrillat spaces of the mid-layer. From these vascular axes capillaries pass out into the country tissue. The capillary can be traced from its origin in an artery out into the mid-layer. Here it forms a loop which eventually brings it back to the vein of the vascular axis from which it started, or may enter another axis, or become lost in the capillary network of an adjacent fat trabecula. In the simplest form it forms a loop and returns to the vein of the axis from which it started. The farthest point of this loop often presents as an acute angle, at the apex of which is a growing capillary. The sides of the loop are connected across by branch capillaries. A series of such ladder-like capillary systems may communicate one with the other, forming a complicated capillary network. As one passes from a vascular axis out into the country tissue the capillaries become more and more rare, so that considerable areas of the mid-layer may be without them.

The nerves do not concern us.

Besides the above-mentioned structures, the mid-layer of the mesentery contains a rich network of connective tissue and elastic fibers. Both these sets of fibers are more numerous about the vascular axes, becoming less numerous as one passes out into the country tissue.

Omentum. — The general structure of the omentum does not differ a great deal from that of the mesentery. The principal point of difference for us lies in the arrangement of the blood supply. As we have seen, the capillary network of the mesentery consists in a collateral circulation appended

to the through vascular trunks. In the omentum, on the other hand, the capillary network is interposed between an afferent artery and an efferent vein.

It has been noted that in experimental peritonitis the exudative phenomenon is more marked in the omentum than in the mesentery. The omentum, owing to its relatively exposed position in the cavity, would receive the first violent effect of the irritant, while the mesentery, more or less protected by the coils of intestine, would be less strongly affected. Again Durham¹⁴ has shown that foreign particles as carmine injected into the peritoneal cavity collect on the omentum, while the mesentery receives but few. In connection with this disproportion in the effects of an irritant on the omentum and mesentery we have been struck with the difference in the arrangement of the capillary blood supply. The blood vessels of the omentum seem to be particularly well arranged to bring the maximum number of blood cells into relation with an intra-peritoneal irritant in the minimum of time. If the irritant acted forcibly for only a short time, as in our experiment, the above facts seem to explain the difference in the amount of exudate in the two situations examined.

VII. CYTOLOGY OF THE MESENTERY AND OMENTUM.

Besides the cells which go to form the vessels and nerves, the mid-layer contains relatively few cells. Those present can be divided into two general classes as follows:

A. Connective tissue cells. These cells are in relation to the connective tissue fibers or lie free. The free connective tissue cells are oval or elongated, frequently with branched processes, and contain a vesicular nucleus. The relation of these cells to the macrophages has been recently discussed by Dominici.

B. Cells which belong to the leucocyte series. Of these cells two sorts are present. First, cells identical with lymphocytes are found in small numbers along the course of the larger vessels, particularly at the bifurcations. Rarely these cells lie in the country tissue. Second, cells identical with the amphophiles of the circulating blood are occasionally

found in the normal mesentery. This is in sharp contrast to the mesenteries of the guinea-pig and rat, which contain many eosinophiles and mast cells respectively.

The cytology of the mid-layer of the omentum differs but little from that of the mesentery. If anything, there are more of the young connective tissue elements or "macrophages en repos."¹²

VIII. DETAILS OF EXPERIMENTS.

TECHNIQUE.

Peritonitis experiments. — The turpentine solution was prepared by shaking one-half cubic centimeter of turpentine with a liter of distilled water. Before filling the syringe for making an injection the fluid was again shaken. The animal was held by an assistant and the hair clipped off over a small area on the abdomen; the skin was then scrubbed with a bit of gauze wet with alcohol. It was found that by gentle handling of the animal all restraint could be dispensed with. By picking up a fold of skin the needle of the syringe could be introduced into the peritoneal cavity without injuring the intestine. When hot salt solution was used for injection the procedure was as follows: About 20 cc. of an 0.8 per cent solution of sodium chloride made up with distilled water was boiled in a test tube with a thermometer. The solution was twice brought to the boiling point and then allowed to cool to 80° C. A sterile hypodermic syringe holding two and a half cubic centimeters was then filled with this fluid and injected. A second syringe full was then injected without reheating the fluid. During the course of the injection the salt solution in the test tube cooled about ten degrees.

In order to exclude animals which were abnormal a preliminary period of fasting was practised in each case. When the animal showed a rising leucocyte count during fasting it was excluded. In all but one case we succeeded in this way in discovering the pregnant rabbits and those with snuffles. The solitary instance of failure we include in the series because it can be used for comparison with the normal cases. (Rabbit 118.)

To rule out secondary bacterial infections we made two cultures from the peritoneal cavity, at autopsy, on coagulated blood serum. After forty-eight hours at 37° C. these tubes were examined and if they showed a growth the animal was excluded.

Leucocyte counts were made from blood obtained by incision of one of the small veins of the ear. Thoma-Zeiss counting apparatus was used. A one-half per cent acetic acid solution was used for dilution. In certain cases the proportion of amphophile leucocytes was determined by making a differential count of the cells as seen on the dilution plate. Throughout one experiment these counts were controlled by differential counts of dried smears and it was found that the number of polymorphonuclears as obtained by the first method was a close approximation to the number of amphophiles obtained by the, perhaps more exact method, of differential count. In tabulating the experiments, the term polymorphonuclear is used when the differential count was made on the dilution plate. When the dried smear is used the different leucocytes are tabulated by name. When dried smears were used they were fixed by heat and stained with Löffler's alkaline methylene blue. In certain cases other stains were used, but for the purpose of a quantitative estimation the simple blue stain was found adequate.

Mesentery and omentum. — To obtain satisfactory preparations of these tissues for cell study the following method was pursued. The fresh mesentery was spread out over the end of a segment of wide calibre glass tubing, or better, the severed neck of a wide mouthed bottle, and tied on by strong thread. The adjacent mesentery and attached intestine was then cut away and the bottle top, with its "drum head" of mesentery, dropped into the fixing fluid. The mesentery remained on the bottle top through all the steps of fixation, hardening and staining until the final clearing with xylol, previous to mounting. A sheet of cigarette paper was then spread over the exposed surface of the mesentery and with a fine pointed scissors a square or circle of mesentery and of paper was cut out. The two were then laid on a slide with

the paper uppermost. One application of a blotter would remove enough xylol from the paper to permit of its being seized with a fine pointed forceps and stripped off the mesentery, leaving the latter spread evenly on the slide. The mesentery was then treated with balsam and covered with a cover glass. By this method all distortion and wrinkling of the mesentery was avoided.

Various stains and fixatives were used, but Zenker's fluid followed by Unna's alkaline methylene blue and eosin was most used, as it gave by far the most satisfactory cell picture.

Bone marrow and other solid organs were fixed in Zenker's fluid imbedded in paraffin by the chloroform method and cut in the Minot ribbon microtome. Sections two clicks in thickness (approximately 6 micra) were found most satisfactory. Unna's alkaline methylene blue and eosin was used for staining. Care was taken to cut sections of the marrow of a uniform thickness. This was done in order that the adult amphophiles of a given area of the section could be counted and so compared with similar counts made upon the marrow of control animals. In order to count the adult amphophile leucocytes in a given area of the marrow an eyepiece with an adjustable, square diaphragm was used. The area of section revealed by the eyepiece was determined by measurement with a stage micrometer. Thus it was possible to count the cells of a known area of the section. The cells of ten such areas were counted in each experiment. Five successive fields were taken at a given distance from the endosteum at one border of the section, and five fields at the same distance from the endosteum forming the opposite border.

EXPERIMENTS.

Rabbit 53. Turpentine peritonitis of two and one-half hours' duration. Female.

Observation I. 1.45 P.M., Aug. 31, 1900.

Leucocytes per cu. mm.	.	.	.	9,000
Mononuclears per cu. mm.	.	.	.	4,900
Polynuclears per cu. mm.	.	.	.	4,100

Put in cage and given no food.

Observation 2. 12 noon, Sept. 1, 1900.

Leucocytes per cu. mm.	8,800
Mononuclears per cu. mm.	6,800
Polynuclears per cu. mm.	2,000

Given an intra-peritoneal injection of 60 minims of a 1-2000 suspension of turpentine, at 12.45 P.M., same day.

Observation 3. 1.15 P.M., same day.

Leucocytes per cu. mm.	8,000
Mononuclears per cu. mm.	5,300
Polynuclears per cu. mm.	2,700

Observation 4. 2.15 P.M., same day.

Leucocytes per cu. mm.	6,200
Mononuclears per cu. mm.	4,400
Polynuclears per cu. mm.	1,800

Observation 5. 3.15 P.M., same day.

Leucocytes per cu. mm.	8,100
Mononuclears per cu. mm.	4,200
Polynuclears per cu. mm.	3,900

Killed by blow on the head at 3.20 P.M., the same day.

Autopsy at once; peritoneum contains a considerable amount of clear fluid. All the organs appear normal in the gross. Mesenteric vessels much injected. Liver extensively infected with coccidia.

Histological Examination.

Mesentery. — The small veins contain many amphophiles which are not evenly distributed. In some places the lumen is almost filled with leucocytes, while over long stretches there will be no more than the normal number present. In the mesentery on either side of the vessels there are many leucocytes which are exclusively of the amphophile type. The leucocytes are not to be found in the mesentery at any great distance from the vessels. In the tissue adjacent to the parts of the vessels containing many leucocytes, the amphophiles are most numerous, and in such areas red blood corpuscles are to be found associated with them. The lymphatics are easily seen, as they contain a large amount of fine granular acidophilic material. No cells are in the lymphatics.

Bone marrow. — The capillaries are well filled with blood. The marrow contains relatively few adult amphophiles and the reticulum is prominent, particularly at the periphery. The dilated capillaries form a prominent feature of the section.

Peripheral blood. — The leucocyte count falls for one and one-half hours after the injection and at the time of killing of the animal it has risen again to where it was before the injection. From the time of the injection till the killing of the animal the mononuclear leucocytes fall steadily. In the same time the polymorphonuclear forms rise. So at the end of the two and one-half hours of the experiment the leucocyte count was the same, but the mononuclears had decreased and the polynuclears had increased. This would seem to indicate that the irritant had caused the lymphocytes to leave the peripheral blood, causing a hypoleucocytosis which was restored to normality by the entrance of polymorphonuclear cell forms.

Rabbit 39. Turpentine peritonitis of six hours' duration. Male, seven to eight months old.

Observation 1. 3 P.M., Aug. 8, 1900.

Leucocytes per cu. mm.	.	.	.	13,100
Mononuclears per cu. mm.	.	.	.	9,300
Polynuclears per cu. mm.	.	.	.	3,800

Put in cage and given no food.

Observation 2. 3 P.M., Aug. 9, 1900.

Leucocytes per cu. mm.	.	.	.	12,300
Mononuclears per cu. mm.	.	.	.	7,500
Polynuclears per cu. mm.	.	.	.	4,800

3.30 P.M. same day, 60 minims of a 1-2000 suspension of turpentine injected into peritoneal cavity.

Observation 3. 4.30 P.M., same day.

Leucocytes per cu. mm.	.	.	.	11,400
Mononuclears per cu. mm.	.	.	.	5,800
Polynuclears per cu. mm.	.	.	.	5,600

Observation 4. 5.30 P.M., same day.

Leucocytes per cu. mm.	.	.	.	11,400
Mononuclears per cu. mm.	.	.	.	4,000
Polynuclears per cu. mm.	.	.	.	7,400

Observation 5. 6.45 P.M., same day.

Leucocytes per cu. mm.	. . .	14,400
Mononuclears per cu. mm.	. . .	5,400
Polynuclears per cu. mm.	. . .	9,000

Observation 6. 7.30 P.M., same day.

Leucocytes per cu. mm.	. . .	17,000
Mononuclears per cu. mm.	. . .	5,800
Polynuclears per cu. mm.	. . .	11,200

Observation 7. 8.30 P.M., same day.

Leucocytes per cu. mm.	. . .	15,300
Mononuclears per cu. mm.	. . .	5,300
Polynuclears per cu. mm.	. . .	10,000

Observation 8. 9.30 P.M., same day.

Leucocytes per cu. mm.	. . .	15,900
Mononuclears per cu. mm.	. . .	6,660
Polynuclears per cu. mm.	. . .	9,300

Animal killed at once. Organs appear normal in the gross.

Summary.—The injection was followed by a moderate hypo-leucocytosis which in turn was followed by a distinct increase above normal. As in Rabbit 53 the hypo-leucocytosis was due to a decrease in the mononuclear forms, which for a time was not offset by the steady rise in the polynuclears. The final increase in the leucocytes was due to increase in the polynuclear forms.

The mesentery showed extensive exudation of amphophiles. The blood within the vessels of the mesentery contained the normal number of leucocytes. In the bone marrow was the same appearance as in Rabbit 53. There is an almost complete disappearance of the adult amphophile leucocytes.

Rabbit 45. Turpentine peritonitis of six and one-half hours' duration. Male, seven to eight months old.

Observation 1. 3 P.M., Aug. 1, 1900.

Leucocytes per cu. mm.	. . .	12,600
Mononuclears per cu. mm.	. . .	6,800
Polynuclears per cu. mm.	. . .	5,800

Put in cage and given no food.

Observation 2. 3 P.M., Aug. 2, 1900.

Leucocytes per cu. mm.	11,000
Mononuclears per cu. mm.	4,900
Polynuclears per cu. mm.	6,100

At 3.15 P.M. the rabbit was given an intra-peritoneal injection of 60 minims of a 1-2000 suspension of turpentine.

Observation 3. 4.15 P.M., same day.

Leucocytes per cu. mm.	6,700
Mononuclears per cu. mm.	2,900
Polynuclears per cu. mm.	3,800

Observation 4. 5.15 P.M., same day.

Leucocytes per cu. mm.	7,000
Mononuclears per cu. mm.	3,200
Polynuclears per cu. mm.	3,800

Observation 5. 6.15 P.M., same day.

Leucocytes per cu. mm.	6,700
Mononuclears per cu. mm.	2,300
Polynuclears per cu. mm.	4,400

Observation 6. 7.15 P.M., same day.

Leucocytes per cu. mm.	11,500
Mononuclears per cu. mm.	4,000
Polynuclears per cu. mm.	7,500

Observation 7. 8.15 P.M., same day.

Leucocytes per cu. mm.	9,200
Mononuclears per cu. mm.	4,000
Polynuclears per cu. mm.	5,200

Observation 8. 9.15 P.M., same day.

Leucocytes per cu. mm.	10,500
Mononuclears per cu. mm.	3,800
Polynuclears per cu. mm.	6,700

The rabbit was killed by a blow on the head at 9.45 P.M., and an autopsy done at once.

The peritoneal cavity contained a small amount of slightly turbid serum. Vessels of the mesentery much injected. Other organs appeared normal in the gross. A count of the white cells in the abdominal fluid showed 20,700 leucocytes per cu. mm. A differential count of the cells counted showed the mononuclear forms to be about 5,800 and the polynu-

clears 14,900. Cultures from the peritoneal fluid remained sterile. The mesentery was preserved in Zenker's fluid and other organs were saved for histological study.

Summary. — The injection was followed by a marked hypo-leucocytosis. This was succeeded by a rise in count, reaching in about six hours to what it was before the injection. Both the mononuclear and polynuclear forms decreased during the hypo-leucocytosis. The subsequent increase was for the most part in the polynuclear cells.

Mesentery. — There was an abundant exudate of the amphophiles, and the blood in the vessels contained leucocytes in the normal proportions.

The bone marrow showed a decrease in the number of adult amphophiles.

Other rabbits received the same injection and were killed after 12, 24, and 48 hours, and 3, 4, 5, 6, and 7 days. There were no notable changes in the leucocyte count after the first twelve hours. After twenty-four hours there was little evidence of peritonitis to be discovered.

The bone marrows could not be distinguished from those of normal rabbits.

Rabbit 109. Hot salt solution peritonitis of three hours' duration. Adult male rabbit. July 8, 1901.

Observation 1. 4.30 P.M., July 8, 1901. Animal feeding.

Leucocytes per cu. mm. . . . 7,200

Differential count:

Lymphocytes, 44.0 per cent . . . 3,200

Large mononuclears, 8.0 per cent . . .

Amphophiles, 45.0 per cent . . . 3,200

Eosinophiles, 1.0 per cent . . .

Mast cells, 2.0 per cent . . .

Given no food.

Observation 2. 11 A.M., July 9, 1901. After 18½ hours' fasting.

Leucocytes per cu. mm. . . . 6,000

Differential count:

Lymphocytes, 65.5 per cent . . . 3,900

Large mononuclears, 1.0 per cent . . .

Amphophiles, 31.5 per cent . . .	1,900
Eosinophiles, 0.5 per cent . . .	
Mast cells, 1.5 per cent . . .	

12 noon. 5 cc. salt solution (sterile) at 75° C. injected into peritoneum.

Observation 3. 1.30 P.M. 1½ hours after injection.

Leucocytes per cu. mm. . . . 4,000

Differential count:

Lymphocytes, 49.0 per cent . . .	2,000
Large mononuclears, 1.5 per cent.	
Amphophiles, 47.0 per cent . . .	1,900
Eosinophiles, 0.5 per cent.	
Mast cells, 2.0 per cent.	

Observation 4. 2.30 P.M. 2½ hours after injection.

Leucocytes per cu. mm. . . . 3,800

Differential count:

Lymphocytes, 20.0 per cent . . .	760
Large mononuclears, 7.0 per cent.	
Amphophiles, 70.0 per cent . . .	2,700
Eosinophiles, 1.0 per cent.	
Mast cells, 2.0 per cent.	

3 P.M., July 9, 1901. Animal killed.

Slight amount of clear fluid in peritoneal cavity.

Analysis of leucocyte counts:

1. During the preliminary period of fasting the total count decreased. The percentage change was —15. This resolves into a decrease in the amphophiles which is only slightly modified by an increase in the lymphocytes.

2. During the first hour and a half after the injection the total count shows a decrease. Percentage change —33 per cent. This resolves into a marked decrease in the lymphocytes. The amphophiles remain stationary.

3. During the next hour following the injection there is a slight decrease. (Not notable in the total count.) Percentage change —5. But this resolves into a considerable further decrease of lymphocytes which is almost compensated for by an increase in the amphophiles.

4. Comparing observation four with two we find that the

aseptic peritonitis has been accompanied by a change in the cell composition of the peripheral blood which can be expressed as follows :

Total count percentage change +36.

Lymphocytes percentage change +80.

Amphophiles percentage change +94.

Histological examination. Omentum.— Both veins, arteries, and capillaries are injected. The capillaries and veins are most intensely injected. The lumina of the veins and arteries are irregular and both present long, fusiform dilatations. This is most marked in the veins. All the vessels are very tortuous. The blood in the arteries contains about the normal number of leucocytes. In the veins are many amphophiles in proportion to the number of red cells. Along the course of the veins the mid-layer about the vessel contains very many amphophiles and red blood corpuscles. The cells are more numerous in certain places than in others. In the cellular collections about the veins the amphophiles are often spherical in form and small. Scattered over the omentum, without relation to the vessels, are areas in which the endothelial nuclei are very numerous. In such areas there are a considerable number of amphophiles, frequently in fantastic shapes. All through the omentum there are many scattered amphophile cells.

The amphophile cells, free in the omentum, present very irregular forms. Some cells show globular projections containing a nuclear mass and connected with the main body of the cell by a very delicate thread of nuclear or protoplasmic material. An occasional lymphocyte and eosinophile cell is to be found.

On the surface of omentum are single, close bundles of rather coarse fibers which overlie the areas in which the endothelial nuclei are numerous. These fibers give the staining reaction of fibrin.

Mesentery.— The vessels are well filled, but show no dilatations. In the arteries the red corpuscles and leucocytes are in normal proportion. In the veins the amphophile cells

are more numerous than normal. In the mid-layer in the neighborhood of the vascular axes the amphophile cells are rather numerous but not so many as in like situations in the omentum. As one passes from the vessels to the portions of the mid-layer more remote from the vascular axis the number of amphophile cells decreases till at a distance they are absent. The lymphatics and nerves are normal. One plasma and several lymphoid cells found along the course of the vessels. The extra vascular amphophiles are in fantastic shapes. At times the amphophiles have masses at a distance from the main body and attached only by a thread of protoplasm.

Bone marrow. Femur.—Amphophile polymorphonuclears few in number. Two hundred and twenty-two found in ten "fields." Other cells appear normal. No evidence of increased proliferation or of degeneration.

Summary.—During the three hours of the peritonitis the blood examinations show an absolute increase in the number of circulating amphophiles. At autopsy many amphophiles are found in an extra-vascular locus at the site of the injection. The bone marrow shows a marked decrease in the number of adult amphophile leucocytes in the sinuses.

Rabbit 113. Hot salt solution peritonitis of three hours' duration. Adult female rabbit. July 12, 1901.

Observation 1. 3.30 P.M. July 12, 1901. Animal feeding.

Leucocytes per cu. mm. . . . 12,700

Differential count:

Lymphocytes, 54.0 per cent . . . 6,900

Large mononuclears, 0.0 per cent.

Amphophiles, 43.0 per cent . . . 5,500

Eosinophiles, 0.5 per cent.

Mast cells, 2.5 per cent.

Kept without food.

Observation 2. 11 A.M., July 13, 1901. After seventeen and one-half hours' fasting.

Leucocytes per cu. mm. . . . 9,400

Differential count:

Lymphocytes, 34.5 per cent . . . 3,200

Large mononuclears, 0.5 per cent.

Amphophiles, 63.5 per cent . . . 6,000

Eosinophiles, 0.0 per cent.

Mast cells, 1.5 per cent.

12.30 P.M., July 13, 1901. Five cc. sterile salt solution at 75° C. injected into peritoneum.

Observation 3. 1.30 P.M. One hour after injection.

Leucocytes per cu. mm. . . . 12,400

Differential count:

Lymphocytes, 28.0 per cent . . . 3,500

Large mononuclears, 0.5 per cent.

Amphophiles, 68.5 per cent . . . 8,500

Eosinophiles, 0.0 per cent.

Mast cells, 3.0 per cent.

Observation 4. 2.30 P.M. Two hours after the injection.

Leucocytes per cu. mm. . . . 5,000

Observation 5. 3.30 P.M. Three hours after the injection.

Leucocytes per cu. mm. . . . 8,000

Differential count:

Lymphocytes, 35.5 per cent . . . 2,800

Large mononuclears, 1.5 per cent.

Amphophiles, 62.0 per cent . . . 5,000

Eosinophiles, 0.0 per cent.

Mast cells, 1.0 per cent.

Animal killed at once.

20-30 cc. of slightly cloudy fluid in peritoneal cavity.

Count of this fluid shows 11,300 "leucocytes" per cu. mm.

The omentum, stomach, and a few coils of intestine are lightly bound together by delicate strands of fibrin. Ap-

pears to be small puncture of coecum. Two blood serum tubes inoculated with peritoneal fluid. No growth after 48 hours at 37° C.

Analysis of leucocyte counts:

1. During the period of fasting the total count decreased. P.C.—26. This resolves into a marked decrease in the number of lymphocytes.

2. One hour after the injection the total count has risen. The percentage change + 32. This brings it back to where it

was before the fasting. The increase resolves into an increase in the number of amphophiles. The lymphocytes remain stationary. Observation four must be excluded from the analysis on account of the absence of the differential count.

3. Three hours after the injection the total count shows a decrease. The percentage change is as follows: II.-IV. — 14 per cent, III.-V. — 35. This resolves into first an increase, then a decrease, in the amphophiles, the lymphocytes remaining about stationary.

4. The total change in the cell composition of the peripheral blood after three hours of peritonitis is very slight. It can be expressed as follows:

Total count, P.C.	. . .	—14 per cent
Lymphocytes, P.C.	. . .	—12 per cent
Amphophiles, P.C.	. . .	—16 per cent

Histological examination. Omentum. — The vessels are well filled with blood. In the large and small veins there are many amphophiles with a few lymphoid and mononuclear cells. Along the course of the smaller veins are numerous areas in which are great accumulations of amphophiles usually with red blood corpuscles. All through the omentum are many amphophiles, but they are not so numerous as about the vessels. Where the amphophile collections are found the number of endothelial nuclei is increased. This is more marked at the distribution of vessels than along main trunks. In the exudate near the vessels "non-granular" leucocytes and lymphoid cells are quite often found. Lying on the surface of the omentum are long bundles of coarse fibrils which take a deep eosin stain (fibrin). In one small arteriole the amphophiles are numerous and are also in the wall and outside. Elsewhere the arterioles are normal. (The cell exudate and diapedesis is much more marked than in rabbit 109.)

Mesentery. — Vessels well filled with blood. The contents of the arteries is normal. In the veins the amphophile leucocytes predominate with a sprinkling of mononuclears and lymphocytes. Along the course of the veins and venules are places where there are accumulations of amphophiles in

the vessel and outside the mid-layer contains many like cells. In most of these areas there are also blood corpuscles and the lumen of the vessel is increased. There are numerous amphophiles along the course of the vessels everywhere. With the amphophile exudate are mixed a considerable number of mononuclear leucocytes and lymphoid cells. The perivascular fibroblasts are fairly numerous and have an abundant protoplasm. As one goes away from the vessels the cells in the mid-layer decrease in number and at a distance only the normal cells (fibroblasts and connective tissue cells) are found.

Bone Marrow. Femur. — Adult amphophile leucocytes are few in number. Ten "fields" contain two hundred and eighty-five. No evidence of degeneration or of increased proliferation found.

Summary. — During the three hours of the peritonitis the blood examinations show no notable quantitative change in the leucocytes. The number of amphophiles shows an insignificant reduction. At autopsy the mesentery and omentum show large extra-vascular accumulations of amphophiles. In the bone marrow the number of adult amphophiles is much decreased.

Rabbit 115. Hot salt solution peritonitis of three hours' duration. Young male rabbit, July 15, 1901.

Observation 1. 4 P.M., July 15, 1901. Animal feeding.

Leucocytes per cu. mm. . . . 8,000

Differential count:

Lymphocytes, 75.5 per cent . . . 6,000

Large mononuclears, 2.5 per cent.

Amphophiles, 21.0 per cent . . . 1,700

Eosinophiles, 0.5 per cent.

Mast cells, 0.5 per cent.

Animal kept without food.

Observation 2. 11 A.M., July 16, 1901. After seventeen hours' fasting.

Leucocytes per cu. mm. . . . 6,800

Differential count:

Lymphocytes, 70.0 per cent . . . 4,800

Large mononuclears, 4.0 per cent.

Amphophiles, 22.5 per cent . . . 1,500

Eosinophiles, 0.5 per cent.

Mast cells, 3.0 per cent.

12.30 P.M., July 16, 1901. 5 cc. of sterile salt solution at 75° C. injected into peritoneum.

Observation 3. 1.30 P.M. One hour after injection.

Leucocytes per cu. mm. . . . 6,400

Observation 4. 2.30 P.M. Two hours after injection.

Leucocytes per cu. mm. . . . 6,100

Differential count:

Lymphocytes, 59.0 per cent . . . 3,600

Large mononuclears, 3.5 per cent.

Amphophiles, 23.0 per cent . . . 1,400

Eosinophiles, 1.5 per cent.

Mast cells, 13.0 per cent.

Observation 5. 3 P.M. Two and a half hours after injection.

Leucocytes per cu. mm. . . . 5,800

Differential count:

Lymphocytes, 54.0 per cent . . . 3,100

Large mononuclears, 2.0 per cent.

Amphophiles, 36.5 per cent . . . 2,100

Eosinophiles, 1.0 per cent . . .

Mast cells, 6.5 per cent.

Animal killed at 3.30 P.M. Three hours after injection.

Peritoneum contains small amount of clear fluid. Vessels well injected in mesentery. Spleen slightly enlarged. Liver somewhat infected with coccidia.

Cultures. — Two on blood serum from peritoneum.

1. Sterile after forty-eight hours at 37° C.

2. Water of condensation contains a short bacillus in chains.

Stains by Gram, no visible growth. Probably a contamination. Not considered grounds for ruling out experiment.

Analysis of leucocyte counts:

1. During preliminary period of fasting the total count decreased. P.C. — 15. This resolves into a decrease in the lymphocytes, the amphophiles remaining stationary.

2. During the first two hours after the injection the total count decreased a little. P.C. — 10. This resolves into a slight further decrease in the lymphocytes, the amphophiles remaining stationary.

3. During the next half hour there is a further slight decrease in the total count. P.C. — 5. This resolves into a slight decrease in the lymphocytes which is almost compensated for by an increase in the amphophiles. The change is complicated by the abnormal number of mast cells in Observation 4.

The effect of the two and a half hours of peritonitis on the cell composition of the circulating blood can be summed up as follows:

Total count, P.C.	—15.
Total lymphocytes, P.C.	—35.
Amphophiles, P.C.	+23.

Histological examination. Omentum. — Vessels well filled with blood. In the arteries the leucocytes and red corpuscles are in normal proportion. In the veins and capillaries the leucocytes predominate. About the vessels and in other areas the endothelial nuclei are more numerous than normal. These areas contain cellular exudate. Except about the vessels and in a few scattered areas the cellular exudate is wanting. In the veins the leucocytes are of the lymphoid, large mononuclear, and amphophile type. Eosinophiles are rarely found either within or without the vessels. In the vessels the lymphoid and mononuclear leucocytes are most numerous. The amphophile cells are numerous, but not predominant. The mononuclear leucocytes can frequently be found in the act of migrating from the vessels. In the immediate vicinity of the vessels the exudate is abundant and is composed of red corpuscles, amphophiles, lymphocytes, mononuclear leucocytes, and eosinophiles. The mononuclears ("non-granulars") are most numerous, the lymphocytes and amphophiles less. The mononuclears and amphophiles show evidence of active ameboid activity.

Mesentery. — The veins and arteries are well filled with the blood. The arteries contain leucocytes and red corpuscles

in normal proportions. In the veins leucocytes predominate. The amphophiles are most numerous, but there are a considerable number of lymphocytes and large mononuclears. In the region about the vessels there are many amphophiles in the mid-layer. Lymphocytes and mononuclears are also present in small numbers. The amphophiles are in fantastic shapes. There is no diapedesis. Associated with the main vascular axis is a large lymphatic which is filled with granular eosinophilic material and contains great numbers of lymphoid cells, also large mononuclear cells like those found in the sinuses of the mesenteric lymph nodes. There are also many cells with "horse-shoe" nuclei and without granules. There are no amphophile cells in the lymphatic vessel. The amphophile cells are not to be found, in the mid-layer, at any great distance from the vascular axes.

Bone marrow. Femur. Adult amphophiles are quantitatively reduced. Ten "fields" contain 206. No evidence of increased proliferation or of degeneration found.

Summary.—The blood examinations during the peritonitis show comparatively little change except that the amphophiles are quantitatively increased. At autopsy the mesentery and omentum show a moderate extravascular accumulation of amphophiles. The bone marrow shows a reduction in the number of amphophiles.

Rabbit 117. Hot salt solution peritonitis of three hours' duration. Adult female rabbit. July 18, 1901.

Observation 1. 4.30 P.M. July 18, 1901. Animal feeding.

Leucocytes, per cu. mm. . . . 5,400

Differential count:

Lymphocytes, 69.5 per cent . . . 3,800

Large mononuclears, 1.0 per cent.

Amphophiles, 27.0 per cent . . . 1,500

Eosinophiles, 0.0 per cent.

Mast cells, 2.5 per cent.

Animal kept without food.

Observation 2. 12 noon. July 19, 1901. After seventeen and one-half hours' fast.

Leucocytes, per cu. mm. . . . 5,300

Differential count:

Lymphocytes, 41.5 per cent . . .	2,200
Large mononuclears, 1.0 per cent.	
Amphophiles, 54.0 per cent . . .	2,900
Eosinophiles, 1.0 per cent.	
Mast cells, 2.5 per cent.	

12.30 P.M., July 19, 1901. 5 cc. of sterile salt solution at 75° C. injected into peritoneum.

Observation 3. 2 P.M. One and a half hours after the injection.

Leucocytes, per. cu. mm.	7,400
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Differential count:

Lymphocytes, 65.0 per cent . . .	4,800
Large mononuclears, 5.5 per cent.	
Amphophiles, 26.5 per cent . . .	1,900
Eosinophiles, 1.0 per cent.	
Mast cells, 2.0 per cent.	

Observation 4. 2.30 P.M. Two hours after injection.

Leucocytes, per cu. mm.	3,000
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Observation 5. 3 P.M. Two and a half hours after injection.

Leucocytes, per cu. mm.	4,100
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Differential count:

Lymphocytes, 40.0 per cent . . .	1,600
Large mononuclears 2.5 per cent.	
Amphophiles, 56.0 per cent . . .	2,300
Eosinophiles, 0.5 per cent.	
Mast cells, 1.0 per cent.	

3.30 P.M. Animal killed. Three hours after injection.

Peritoneal cavity contains small amount (20 cc.) of slightly reddish cloudy fluid. Fluid contains 15,000 "leucocytes" per cu. mm. Mostly polymorphonuclears. All organs appear normal in the gross. Bacteriological examination.

The cultures on blood serum from peritoneal cavity show no growth after forty-eight hours at 37° C.

Analysis of leucocyte counts:

1. During the preliminary period of fasting the total count

shows little change. P.C. — 2. This resolves into a decrease in the lymphocytes which is compensated for by an increase in the amphophiles.

2. During the first hour and a half after the injection the total count shows an increase which amounts to P.C. +40. This resolves into an increase (!) in the lymphocytes which is in part modified by a decrease (!) in the amphophiles.

3. During the succeeding hour the total count decreases considerably. P.C. —45. This resolves into a decrease in the lymphocytes, the amphophiles remaining about the same.

4. The total effect of two and a half hours of peritonitis on the cell composition of the peripheral blood can be expressed as follows:

Total count, P.C.	—22
Lymphocytes, P.C.	—27
Amphophiles, P.C.	—20

Histological examination. Omentum. — Vessels well filled with blood. Contents of arteries normal. In the veins and venules the amphophiles are by far the most numerous cells. Along the course of the vessels is an abundant exudate of amphophiles with which are a few large mononuclear cells and an occasional lymphocyte. The extravascular amphophiles show evidence of active amœboïd motion, and in some cases seem fragmented. The cell exudate is confined to the vicinity of the vessels and the increase of endothelial nuclei is not marked. The endothelial nuclei are numerous about the vessels, and small scattered areas are found away from the vessels. In these areas, which are remote from the vessels, the amphophile exudate is also present.

Mesentery. — The vessels are well filled with blood. The contents of the arteries is normal. In the veins the predominant cell is the amphophile, although lymphoid and large mononuclear cells are also present in small numbers. In the mid-layer of the mesentery are many leucocytes, almost all of which are amphophiles. An occasional lymphoid and large mononuclear cell are found. The amphophiles are most numerous along the course of the vessels.

There are many foci of diapedesis from the small veins. The amphophiles are not in such fantastic shapes as usual. The lymphatics are normal.

Spleen.—The Malphighian bodies and vessels are normal. In the pulp the cells lining the sinuses are increased in number. Polynuclear amphophiles are numerous. One nucleated red corpuscle seen. There are a considerable number of giant cells like those of the bone marrow. One of these seems to lie in a small capillary. One myelocyte found with an indented nucleus and amphophile granules in protoplasm.

Bone marrow. Femur.—Ten "fields" contain two hundred and thirty-five.

Adult amphophiles quantitatively reduced.

No evidence found of an increased proliferation or of a degeneration.

Summary.—The blood examinations during the peritonitis show a slight quantitative decrease in the number of amphophiles.

At autopsy the mesentery and omentum show a considerable extra-vascular accumulation of amphophiles. The bone marrow shows a quantitative decrease in adult amphophiles.

Rabbit 118. Adult female rabbit. July 20, 1901.

Observation 1. 5 P.M., July 20, 1901. Animal feeding.

Leucocytes per cu. mm. . . . 10,100

Differential count:

Lymphocytes, 38.5 per cent . . . 3,900

Large mononuclears, 0.5 per cent.

Amphophiles, 59.0 per cent . . . 6,000

Eosinophiles, 0.5 per cent.

Mast cells, 1.5 per cent.

Animal kept without food.

Observation 2. 11.30 A.M., July 21, 1901. After seventeen and one-half hours' fasting.

Leucocytes per cu. mm. . . . 7,800

Differential count:

Lymphocytes, 55.0 per cent . . . 4,300

Large mononuclears, 3.0 per cent.

Amphophiles, 40.0 per cent . . . 3,100

Eosinophiles, 0.5 per cent.

Mast cells, 1.5 per cent.

12 noon. 5 cc. of sterile salt solution at 75° C. injected into peritoneum.

Observation 3. 12.30 P.M. Half hour after injection.

Leucocytes per cu. mm. . . . 10,000

Observation 4. 1.30 P.M. One and a half hours after injection.

Leucocytes per cu. mm. . . . 4,400

Differential count:

Lymphocytes, 31.0 per cent . . . 1,400

Large mononuclears, 1.5 per cent.

Amphophiles, 66.0 per cent . . . 2,900

Eosinophiles, 0.0 per cent.

Mast cells, 1.5 per cent.

Observation 5. 2.30 P.M. Two and a half hours after injection.

Leucocytes per cu. mm. . . . 5,700

Observation 6. 3 P.M. Three hours after injection.

Leucocytes per cu. mm. . . . 10,000

Differential count:

Lymphocytes, 25.5 per cent . . . 2,600

Large mononuclears, 1.0 per cent.

Amphophiles, 73.0 per cent . . . 7,300

Eosinophiles, 0.0 per cent.

Mast cells, 0.5 per cent.

3 P.M. Animal killed.

Peritoneum contains about 20 cc. of slightly cloudy fluid with a reddish tinge in which float fine flakes of fibrin. Bone marrow appears redder than normal. Other organs appear normal with the exception of a small area upon one lung.

Peritoneal fluid contained 9,000 "leucocytes" per cu. mm.

Bacteriological examination. — Two cultures on blood serum from peritoneal fluid. Sterile after forty-eight hours at 37° C.

Analysis of leucocyte counts:

1. During the preliminary period of fasting the total

count decreased. P.C. —22. This resolves into a decrease in the amphophiles which is partly compensated for by a slight increase (!) in the lymphocytes.

2. During the first hour and a half after the injection the total count shows a marked decrease. P.C. II.—IV.—56. This resolves into a marked decrease in the lymphocytes and a slight decrease in the amphophiles.

4. During the next hour and a half the total count increased. P.C. IV.—VI. +120. This resolves into a marked increase in the amphophiles and a slight increase in the lymphocytes.

The total change in the cell composition of the peripheral blood during the three hours of peritonitis can be expressed as follows:

Total count, P.C.	+28
Lymphocytes, P.C.	—39
Amphophiles, P.C.	+135

Omentum. — Vessels well filled with blood. The contents of the arteries are normal. In the veins leucocytes predominate. There is an abundant cellular exudate all through the omentum. The cells are most numerous about the vessels and in small areas away from the vessels. Where the cells of the exudate are most numerous there are an increased number of endothelial nuclei. In the veins there are many amphophile cells and almost as many lymphoid cells. There are also a considerable number of large mononuclear cells. In the exudate away from the vessels the cells are all amphophiles, while near the vessels there are a considerable number of lymphoid and large mononuclear cells. Branched fibroblasts are numerous.

Mesentery. — The vessels are only moderately filled with blood and the leucocytes are not markedly increased in number. Along the course of the vessels there are a moderate number of amphophiles in the mid-layer. An occasional large mononuclear and lymphoid cell is found. There are few amphophiles at any distance from the vascular axes. The process as shown in this preparation is very slight compared with the corresponding omentum.

Lung. — Along one border the lung vesicles under the pleura are collapsed, and in this region the number of amphophiles is considerable. The amphophiles are in the capillaries and also in the collapsed air cells. In certain places the air cells contain large cells with a finely granular protoplasm, and a vesicular nucleus, some of which are phagocytic for the amphophiles. In other parts of the lung the amphophiles are not increased in number.

Summary. — The blood examinations during the peritonitis show an increase in the number of amphophiles.

At autopsy the mesentery, omentum, and peritoneal fluid show a considerable extra-vascular accumulation of amphophiles. The bone marrow shows a quantitative decrease in the adult amphophiles. The acute inflammatory condition in the lung may account for the somewhat greater number of amphophiles noted in the blood and bone marrow than in the other animals of this series.

CONTROL RABBITS.

In order to determine what effect fasting, feeding, pregnancy, and snuffles might have upon the cell richness of the bone marrow in amphophiles the following observations were made:

Rabbit 111. Adult female. Animal killed after five hours' feeding. All organs appear normal. Sections of bone marrow of femur prepared like those from peritonitis rabbits show 637 adult amphophiles in ten "fields."

Rabbit 112. Same as rabbit 111. Ten "fields" in bone marrow show 556 adult amphophiles.

Rabbit 124. Adult male. Allowed to fast for twenty-four hours before killing. All organs appear normal. Bone marrow shows 554 adult amphophiles in ten "fields."

Rabbit 116. Adult. Animal showed marked symptoms of purulent rhinitis for ten days. Fed for five hours before killing. At autopsy nostrils showed typical lesions of "snuffles." Bone marrow of femur contained 731 adult amphophiles in ten "fields."

Rabbit 119. Adult female. Animal tested twice by

leucocyte counts before and after a period of eighteen and one-half hours' fasting. In each case the leucocyte count showed a marked rise. After second trial animal showed symptoms of snuffles. Killed after twenty-two hours' fasting.

Protocol. — In left nasal cavity, lying above the hard palate and near the septum, is a collection of white muco-purulent material.

Mucous membrane injected. On the anterior portion of the upper lobe of the right lung is an area of firm consistency and a brownish red color. The area is sharply marked off from the remainder of the lung, which is light pink and crepitant. Similar areas lie at and near the apex of the left lung. Peritoneal cavity contains slight amount of clear fluid.

Microscopic examination. *Lung.* — With the exception of a small corner of the section the air cells are almost obliterated. In the obliterated area the vessels are much injected and contain many amphophiles and a considerable number of eosinophiles. In one place the eosinophiles are in a vein, in its wall, and in the peri-venous areolar tissue. In the obliterated air cells the cells lining the cavities are very large and have finely granular protoplasm. Many such cells lie free in the air spaces. These cells are markedly phagocytic.

The amphophile cells are numerous both in the vessels and in the air spaces. In certain areas there is a focal necrosis and there the amphophiles and epithelial cells are very numerous. In such areas the cells are partly necrotic and there are many chromatin fragments. These necrotic areas lie under the pleura. In the perivascular lymph sinuses there are many lymphoid and plasma cells.

The process seems to be chiefly proliferative with an exudative process accompanying. The presence of both amphophiles, eosinophiles, and lymphoid cells associated with the lesions suggests that it is sub-acute or chronic in character.

Section of bone marrow from femur prepared as before showed 695 adult amphophiles in ten "fields."

Rabbit 123. Adult female. Animal allowed to fast for eighteen hours. At autopsy found to be in early stage of pregnancy. Bone marrow prepared as before showed 758.

Rabbit 125. Adult female. Animal allowed to fast for eighteen hours before killing. Section of marrow from femur, prepared as before, shows 549 adult amphophiles in ten "fields."

Rabbit 126. Adult female. Animal allowed to fast for twenty-four hours before killing. At autopsy found to be pregnant. Embryos 5 cm. long. Section of bone marrow from femur, prepared as before, showed 532 adult amphophiles in ten "fields."

A comparison of the determinations of cell richness of the marrow in adult amphophiles yields the following: An area of section of 28 sq. mm. was searched in each marrow and all the adult amphophiles there were counted. (See p. 27.)

Hot salt solution peritonitis rabbits, 222, 285, 206, 235, 386. Maximum, 386; minimum, 206; average, 267.

Control rabbits. (Feeding, fasting, snuffles, pregnancy.) 637, 556, 731, 554, 758, 549, 532, 695.

Maximum, 758; minimum, 532; average, 626.

IX. SUMMARY OF EXPERIMENTS.

From the foregoing data we find that during the early stages of a mild peritonitis, induced by the injection of dilute suspension of turpentine, the amphophile leucocytes accumulate in the vessels of the mesentery and emigrate into the extra-vascular tissue; that during this time the number of amphophile leucocytes in the peripheral blood suffers a diminution, followed by an increase; that THE BONE MARROW BECOMES DEPLETED OF ADULT AMPHOPHILE LEUCOCYTES.*

When hot salt solution is used to cause the peritonitis the phenomena were essentially the same; but in two instances (Rabbits 113 and 117) the number of circulating

* Muir notes a decrease in amphophiles in the marrows of rabbits killed during the leucopenia following bacterial injection. Rubinstein found a similar condition in his experiments.

amphophiles does not show an increase. The mesentery and bone marrow showed the same changes as before.

The cell richness of the marrow in adult amphophiles is not notably affected by a short period of fasting, by feeding, by pregnancy, or by a relatively chronic infection with "snuffles."

X. DISCUSSION.

With the data at hand we can now formulate certain conclusions with respect to the problems outlined in the beginning of this paper.

I. As to the locus of origin of the amphophile leucocyte in the rabbit. The results obtained by the experiments, taken together with the morphological studies of the cells of the marrow, are confirmatory of the view, already widely held, that the bone marrow is the main source, if not the only source, of the circulating amphophile leucocyte.

II. As to how the supply of circulating amphophile leucocytes is maintained we believe our data sufficient to formulate a hypothesis. In so doing we will not discuss the theories of leucocytosis which have been advanced from observations of the numerical variations in the leucocytes in the circulation under the influence of various injections. We wish rather to base our hypothesis upon the correlation of the changes to be found in the bone marrow and in the circulating blood under experimental conditions. Work of this kind has been done by Ribbert, Muir, Roger and Josué, Taylor, and Rubinstein.

Rubinstein has given a most excellent account of the process of leucocyte production in the marrow, and we agree fully with him in the morphological aspects of the problem. The recognition of the fact that the amphophiles come about by a series of metamorphoses from an undifferentiated cell always resident in the marrow is of the greatest importance. Minot¹⁵ divides tissues into two classes, in one of which there are cells which concern themselves rather with the primitive function of multiplication and from which specialized cells arise, both sorts of cells, however,

being always present in the tissue. The bone marrow must be conceived of in this light to render the phenomena of leucocyte supply understandable. So much for the morphological aspects of the problem. We now come to the more speculative side of the question. How is the supply of adult circulating amphophiles maintained?

We have a highly specialized cell circulating through the vessels, we know that these cells are constantly being lost through passing out of the blood stream and even out of the body as by emigration through mucous surfaces. To a less extent a loss is doubtless occasioned by the breaking down of individual cells in the blood stream, and by their being engulfed by other cells.

Evidence is lacking to show that emigrating leucocytes return directly to the vessels, the cell contents of the thoracic duct, as Muir states, are overwhelmingly non-granular, so few can get back via the lymphatics. There remains the alternative of multiplication in the blood stream or a constant inflow of new cells to account for the maintenance of the supply. For the first of these sources of supply, multiplication in the blood stream, two mechanisms are conceivable: first, intra-vascular metamorphosis; second, multiplication by direct or indirect division. As to these possibilities we must side with Muir, who emphatically denies their existence as a factor in leucocytogenesis. So far as we are aware there are no authentic observations on mitosis in amphophile leucocytes in the circulation of the rabbit. In human blood it is worthy of note that mitoses are only found in disease in which myelocytes might be expected in the circulation, *e.g.*, leukemia or severe infections.

We are therefore forced to conclude that the supply of amphophile leucocytes in the circulation owes its origin to the entrance of fully formed cells from an extra-vascular locus. The morphological evidence is conclusive that in the rabbit the marrow, at least under normal conditions, is this locus.

This brings us to the consideration of the mechanism of the supply. Obviously we have here to do with two processes. First, that which has to do with the mere movement of

the adult amphophile from its extra-vascular locus of origin in the marrow to its normal adult habitat in the blood stream. Second, that which has to do with the production of the adult amphophile from the undifferentiated cell of the marrow.

The first of these processes, which can be briefly designated as motor, evidently comes to pass either by passive extrusion, as by the pressure of accumulated cells and the like, or by active locomotion of the individual cell under the influence of external stimulus. The much studied phenomena of chemotaxis seem to fully account for this act of motion on the part of the leucocyte. We can reasonably suppose that the chemical composition of the blood serum is such that it presents varying degrees of chemotaxis for the leucocytes. We must conceive of its always being, in the state of health, to some degree chemotactic towards the leucocytes.

If we assume that our second part of the mechanism of leucocyto-genesis is operative and that we have a constant supply of adult leucocytes in the sinuses of the marrow, that is, that cell differentiation is going forward, we can explain variations in the number of circulating amphophiles by conceiving of the blood as presenting varying degrees of chemotaxis. A leucocytosis is then only an indication of a change in the chemical composition of the blood; the change involving an accentuation of the chemotactic index. The maintaining of the normal number of circulating amphophiles will therefore depend in part upon the maintenance of a greater or less chemotactic index in the blood which will draw new amphophiles from the marrow to balance the losses of individual circulating leucocytes. To cite an example, we know that the products of certain bacterial growth, when mixed with the blood, cause leucocytosis. It is not inconceivable that the chemotactic index of the blood and therefore the maintenance of the supply of circulating leucocytes depends upon the absorption of such products from the bacteria which flourish in the enteric tract. Again we know that the products of cell destruction act in a similar

way. Hence the chemotaxis of the blood may depend in part upon the metabolic products inherent to the activities of the tissues in general. As these substances have been shown capable of causing purposeful motion in leucocytes by direct action, we need not have recourse to a more complicated process to explain this motion of the leucocytes from the marrow to the blood. Granted the entrance of such substances into the circulation, the mere change of locus of the adult amphophile from the marrow sinus to the blood stream is readily understood.

Turning now to the second process in the amphophile supply, the differentiation of the adult amphophile from the undifferentiated marrow cell, we confront a fundamental problem. We have morphological evidence that an individual amphophile has undergone a series of metamorphoses which lead from an undifferentiated cell, a cell of much less structural specialization, probably much less functional specialization, and much greater potentiality for specialization. This process goes on in the adult marrow, and the supply of adult circulating amphophiles is dependent upon it.

Bound up with this process of cell production is another factor which also plays an important part in the maintenance of amphophile supply. That is cell multiplication. Each undifferentiated cell of the marrow does not give rise to one but to many amphophile leucocytes, for as specialization goes forward it is interrupted by mitosis. Any factor which causes mitosis in any of the cells of the series will have an important bearing upon the question of leucocyte supply.

In some instances we find that experimentally introduced substances must be considered as possible factors in the maintenance or increase of leucocyte production in the marrow. Repeated experiments have demonstrated that continued injection into the body of certain substances will bring about and maintain an increase in the number of circulating amphophiles, and with this appears a hyperplasia of the marrow whereby it becomes crowded with myelocytes. To us this phenomenon is to be regarded as a simple proliferation of myelocytes, not as an exaggeration of the process of leuco-

cyte specialization in the marrow. We have compared such a marrow with the normal in the following way: A normal rabbit was given ether, and a window cut in the left tibia so that a bit of marrow could be removed. This bit of marrow was fixed, sectioned, and stained. The rabbit was given several injections of a bouillon culture of *B. mucosus capsulatus*. At the end of a week the animal was killed and the bone marrow from the right tibia, at the same level as that removed from the left, was fixed, sectioned, and stained. Previous experiments had shown that in the normal animal the marrow of corresponding levels in the two tibiae are practically identical. The marrow removed at operation was normal in every respect, showing the characteristic diversity of cell contents. The marrow removed at autopsy showed the picture of a leucoblastic marrow. The myelocytes were by far the most prominent cells and present numerous mitoses. This marrow picture is explainable as an example of proliferation in the myelocyte under the influence of a bacterial toxin. It does not necessarily prove that the process of cell differentiation was accelerated. Quite the contrary, for if this were the case the diversity of cells in the marrow would be greater. That is, if increased differentiation kept pace with increased proliferation, the numerical relations of the cells of the amphophile series would be maintained. The fact that the myelocyte must undergo further specialization before entering the blood as an adult amphophile does not make it necessary that a substance which causes proliferation of the amphophile myelocyte and migration of the adult amphophile from the marrow and the blood stream shall also preside over the specialization of the myelocyte into the adult leucocyte. In the normal state such a specialization is always going on and we have no reason to think that the amount of the causation factor which in health brings about the specialization of the myelocyte into the adult amphophile would be insufficient to cause the specialization of the increased number of myelocytes that are produced by the action of the toxine. Bacterial toxins can therefore be considered as factors in the motion of adult amphophiles from the marrow to the blood

and as excitants to proliferation of the myelocytes, but we must seek further for the causative agent for the leucocyte differentiation in the marrow.*

In the normal animal this cell specialization goes forward, and if it is dependent upon influence brought to it from without the marrow it is conceivable that it depends upon the action of other body cell products upon the marrow cells. It is equally conceivable that the process is inherent to the cell and is a phenomenon of heredity.

XI. CONCLUSION.

1. We recognize the bone marrow as the principal locus of origin for the amphophile leucocyte of the rabbit.

2. We conceive of the supply of amphophile leucocytes to the blood as under the control of three factors:

I. Chemotactic, which have to do with the movement of adult amphophiles from the marrow to the blood stream.

II. Differentiative, which have to do with the differentiation of the undifferentiated marrow cells into the adult amphophile.

III. Proliferative, which cause mitosis in the marrow cells of the amphophile series, particularly the myelocytes.

In short, the phenomenon of amphophile supply and of amphophile leucocytosis on analysis is divisible into cell motion, cell differentiation, and cell multiplication.

In the experiments here detailed we have sought to make prominent the motor phenomenon.

Normal marrow with its diversity of cell contents shows particularly the phenomenon of cell differentiation.

The hyperplastic marrow of continued leucocytosis emphasizes the part played by cell multiplication.

(NOTE. — We wish to express our thanks to Dr. W. T. Councilman for his assistance and encouragement in this work).

* In this explanation of the leucoblastic marrow we lean to the view that the increase in the myelocytes is dependent upon proliferation under the influence of a toxine, according to the general law outlined by Mallory,¹⁷ rather than to over-regeneration after loss. (Weigert's doctrine.)

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ON THE USE OF CLAY MODELS TO RECORD MUSCULAR
VARIATIONS FOUND IN THE DISSECTING ROOM, WITH
A NOTE ON TWO CASES OF M. STERNALIS AND ITS
INFLUENCE ON THE GROWTH OF M. PECTORALIS
MAJOR.*

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During the past three years it has been our custom to record the interesting muscular variations found in the dissecting rooms by means of clay models. This work is done by the students, all of whom have attained more or less proficiency in the method during the preliminary work in osteology. Here in the regular course they are required to model all of the bones of the body. In watching the work of a class one is impressed on the whole with the quality of the models produced, and the relatively high standard maintained by an average student. Every one, apparently, carries in his sensorium a very good stereognostic sense which is easily developed by a little training. The value of the model as a record is apparent, for the relations, form, and size of the muscle under consideration together with its nerves and blood supply can be recorded in plastic form just as they are found in the subject. To the student the advantage of the method lies in the fact that he registers not only repeated visual images necessitated by the close study required to make a replica, but also utilizes his tactile sense and muscle sense as well. The superiority of the modelling over drawing lies in the fact that arbitrary laws of perspective are not required, and the student has the advantage of having his record of the object in three dimensions just as it exists in nature. For preservation, the models can be shellacked and then colored with ordinary enamel paints, making remarkably realistic representations of the original

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specimens. It is possible, moreover, to utilize them for publication by the simple photographic methods, and finally they may become valuable additions to the museum or the laboratory.

The following brief account of two cases of *M. sternalis* found in the dissecting rooms during the past two years may serve to illustrate the points we have just discussed:

CASE I.

The muscle (Figure 1) appeared in the body of a negress, aged about thirty-two years. It was found in the dissecting rooms of the University of Chicago.

On the right side the muscle is broad and thin. It arises from two heads, the median about one and one-half centimeters and the lateral two centimeters broad, from tooth-like tendinous processes on the surface of the pectoral muscles just over the costo-sternal junction of the third rib. The muscle is not more than one to two millimeters in thickness, curves outward from the mid-sternal line and then inward to become inserted on the sheath of the rectus abdominis. Just above the point of insertion the two heads fuse and are attached together. The medial portion of the muscle is inserted by tooth-like tendinous processes like the origin, while in the lateral half the muscle fibers lose themselves in the sheath of the rectus. The muscle is made up of broad fasciculi which are separated by little bands of fascia. In this case the nerve supply of the muscle was lost through careless dissection, but the blood supply came through the perforating rami of the internal mammary artery. On the left side the muscle is represented by a single lanceolate-shaped slip arising by a little tendinous process from the left pectoralis major about the middle of the third interspace and the costo-sternal junction of the fourth rib. It curves slightly outward and then inward, and terminates by a short tendinous insertion into the sheath of the rectus on the left side. The presence of these muscles has had a very remarkable effect on the growth of the great pectorals. The *M. sternalis* on the right side has inhibited the growth of the right *M. pec-*

toralis major, so that its point of origin is some three and one-half centimeters from the m. s. l. Cephalad of the variable muscle, the M. pectoralis major has its normal origin. The influence on the medial growth apparently is exerted only on the portion covered directly by the M. sternalis. The pars abdominalis of the pectoralis major is separated from the pars sternalis by about two centimeters. The presence of the small slip on the left side has had no inhibiting influence on the growth of the left pectoral muscle, for this, after reaching its normal line of origin, has passed beyond it and arises well to the right side of the sternum.

CASE II.

The M. sternalis appeared in a well-developed negro between twenty-five and thirty years of age. The muscles are placed beneath the deep layer of superficial fascia on either side of the mid-sternal line. That on the right side is larger and better developed than the one on the left. The right muscle possesses a tendon at the cephalic extremity which is continuous with the sternal insertion of the left sterno-cleido-mastoid. Certain fibers also bifurcate and pass into the insertion of the right sterno-cleido-mastoid, forming a Y-shaped tendon. The shape of the right sternalis muscle is lanceolate. It is four centimeters in width at a point above the third costal cartilage, and is one and eight-tenths centimeters wide at its lower extremity. The cephalic end is tendinous, and this structure extends into the middle of the flat belly of the muscle to a point midway between the second and third costal cartilages. The lower extremity is muscular almost to its point of insertion, where a few tendinous fibers are given off. The muscle curves with its convexity away from the mid-sternal line. It is penetrated by the anterior perforating branches of the first, second, third, and fourth intercostal nerves. It receives its blood supply from the accompanying arteries. The insertion is at the costo-sternal junction of the sixth rib. A little tendinous slip passes from the fascicles of the left pectoralis major to the tendon on the right sternal muscle at a point two and one-half centimeters from the

sternal notch. The right sternal muscle is smaller, measuring approximately two and two-tenths centimeters at the point of its greatest breadth. The cephalic extremity is tendinous and begins at a point on the right half of the sternum about one centimeter above the costo-sternal junction of the second rib, where the flattened tendon is overlaid by the *M. pectoralis major*. The insertion is smaller and its tendinous fibers lose themselves on the surface of the sternum just above the attachment of the fourth rib. The muscle is penetrated by the anterior perforating branches of the second and third intercostal nerves. Blood vessels that accompany these branches enter the muscle and supply it. The left *pectoralis major* is attached nearer the mid-sternal line than the right. The portion directly over the first interspace passes the median line and interdigitates with a similar portion of the right *pectoralis major*. This part is overlaid by a few fascicles of the latter muscle which originate directly from the tendon of the right *M. sternalis*. The presence of these two variations has also had an influence on the development of the two pectoral muscles. With the exception of the interdigitation mentioned above, the portions of the *pectoralis major* on either side extending from the sternal notch to the second rib meet at the mid-sternal line. The right *pectoralis major* has been inhibited in its growth medianward by the presence of the *sternalis* muscle above, making its point of origin in this region one and one-half centimeters from the mid-sternal line in the second interspace, two and one-half centimeters in the third, and two and one-fourth centimeters in the fourth interspace.

We are all familiar, of course, with the discussion concerning the morphological significance of the *M. sternalis* and the various views that have been advanced to explain its presence. That it is a muscle peculiar to man is held by Halberstema, while others have maintained that its homologue exists in the lower series. Bourienne and Marjolin, for example, think that it is an extension of the *M. sternocleido-mastoideus*. Poirer¹ states that Lavocat believed it to

¹ Poirer et Charpy. *Traité d'Anatomie Humaine*, T. ii., Paris, 1901.

be a dissociated portion of *M. pectoralis major*, while Bardeleben¹ differentiates two forms, one a variety of the *pectoralis major* and the other the true *sternalis*, an atavistic derivative of the *pubohyoid* or *rectus abdominis* group of muscles. Christian,² who has recently reviewed the subject in reporting two cases, agrees with the view of Von Bardeleben. Clearly both of these cases are members of the second group, although in one of them the nerve supply was not determined. Attention has not been called, however, to one significant fact associated with their presence, and that is the effect they produce on the growth of the great pectorals, as well as the influence exerted by the growth of one pectoral on the other. In both cases the symmetrical origin of these large girdle muscles has been effected by the presence of the muscular sheaths above them. From these specimens it appears that if the *Mm. sternales* are of sufficient size they can effect the growth of the pectoral muscles medianward. As is shown by the left *sternalis* in Case I., however, it may be too small to have any influence on the wandering of the pectoral, but the behavior of the two pectorals in this instance suggests that growth is restricted at the mid-sternal line by the opposing forces of the two muscles, which neutralize each other and cause their arrest at the mid-sternal line. If, however, for any reason such as the presence of a *sternalis* muscle, this tendency is inhibited, then under such conditions, the opposing pectoral may overgrow its normal point of origin and encroach on the field usually occupied by its symmetrical fellow. This may perhaps be extended so as to cover other structures which reach the mid-line, although instances of this kind have not as yet been reported. It seems, therefore, that the median plane of symmetry established on the muscular portion of the ventral thoracic wall depends partly on the equal growth of symmetrical structures from opposite sides of the body, and if, for any reason, this force is withdrawn, the opposing structure still retains suffi-

¹ Bardeleben. *Anat. Anzeiger*, Bd. iii., 1888.

² Christian. *Bulletin of the Johns Hopkins Hospital*, 1898.

cient power of growth to encroach upon territory where it does not normally belong.

PLATE XXII.

FIG. 1. Clay model of Case I. Shellacked and painted with enamel paints. x $\frac{1}{2}$.

FIG. 2. Clay model of Case II. Same preparation as Case I. x $\frac{1}{2}$.

A STUDY OF THE DISTRIBUTION OF THE COLON BACILLUS
OF ESCHERICH AND OF THE SEWAGE STREPTOCOCCI
OF HOUSTON IN POLLUTED AND UNPOLLUTED WATERS.

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Contents: 1. Previous publications bearing on the distribution of the Colon Bacillus in water. 2. Methods of analysis pursued in this investigation. 3. Examination of one hundred and fifty-seven samples of water from apparently unpolluted sources. 4. Examination of fifty samples of water from obviously polluted sources. 5. Conclusions.

1. PREVIOUS PUBLICATIONS BEARING ON THE DISTRIBUTION OF THE COLON BACILLUS IN WATER.

Ten years ago the *B. coli* of Escherich occupied a position of very great prominence in the eyes of sanitarians. If it was not considered to be in itself a dangerously pathogenic germ, it was at least regarded as a suspiciously close relation of the typhoid organism. At this time, therefore, when the alleged presence of either of these forms was quite sufficient to condemn a water supply, the main processes for their isolation were first worked out. All our modern methods still depend either upon the incubation of water at a high temperature with the addition of phenol, as suggested by Vincent in 1890,¹ or upon the inoculation of a solid medium containing sugar and litmus, as recommended a little later by Wurtz.² It was in 1890 also that Dr. Theobald Smith introduced the use of the fermentation tube into bacteriology;³ and in the next few years he established the great importance of its application, both to the separation of the varieties of the colon group and to the quantitative estimation of the representatives of that group present in water.^{4,5}

Investigation soon showed, however, that the *Bacillus coli* was by no means confined to the human intestine. Dyar and Keith⁶ found it to be the prevailing intestinal form in the cat, dog, hog, and cow. In the goat and rabbit they re-

ported that no single organism was constantly present; and in the case of the horse, the place of the colon bacillus was taken by a new form, described by the authors under the name of *B. equi intestinalis*. About the same time, Fremlin⁷ found colon bacilli in the feces of dogs, mice, and rabbits, but not in those of rats, guinea-pigs, and pigeons. Smith⁸ recorded the presence of the same organism, in almost pure cultures, in the intestines of dogs, cats, swine, and cattle; and he also found it in the organs of fowls and turkeys after death. Brotzu⁹ reported *B. coli* and allied forms as very abundant in the intestine of the dog; and Belitzer,¹⁰ still more recently, isolated typical colon bacilli from the intestinal contents of horses, cattle, swine, and one goat. In this country two communications have been lately made upon the subject. Russell and Bassett¹¹ stated that studies made by E. B. Hoag in Professor Russell's laboratory indicated the presence of bacilli of the colon group, fermenting dextrose with a gas formula of 2-1, in the feces of "a considerable number of different species of mammalia, as well as that of birds, fish, etc.," while similar organisms with the inverted gas formula 1-2, were considered by the same authors to be characteristic of decomposing organic matter of vegetable origin, free from suspicion of fecal contamination. Finally, Moore and Wright¹² recorded the finding of the colon bacillus in the horse, dog, cow, sheep, and hen; and in a later report¹³ they noted its occurrence in swine and in some but not all the specimens of rabbits examined. In frogs it was not found.

Many bacteriologists have gone much further, and affirmed that the colon bacillus was not a form characteristic of the intestine at all, but a saprophyte having a wide distribution in nature. The first of this school, perhaps, was Kruse¹⁴ who in 1894 protested against the arbitrary conclusions drawn from the colon test as then applied. He pointed out that the characters usually observed marked not a single species but a large group of organisms. As ordinarily defined, he added, "the *Bacterium coli* is in no way characteristic of the feces of men or animals. Such bacteria occur everywhere,

in air, in earth, and in the water, from the most different sources." Even if the relations to milk and sugar media be considered, "micro-organisms with these characteristics are also widespread." Dr. Kruse gave no experimental data on which his opinion was based. In the same year Beckmann¹⁵ isolated a bacillus which he identified by pretty thorough tests as *B. coli* from the city water of Strassburg, a ground water which he believed could by no possibility be subject to fecal contamination. Large quantities of water were used for the isolation.

Refik in 1896¹⁶ recorded the constant presence of colon bacilli in water of all sorts, public supplies, wells, cisterns, and springs, in the neighborhood of Constantinople. He distinguished five types of "colon bacilli," as follows: Type A fermented dextrose and lactose, coagulated milk, did not form indol; type B fermented dextrose and lactose, did not coagulate milk, did form indol; type C fermented dextrose and lactose, did not coagulate milk or form indol; type D coagulated milk, did not ferment either lactose or dextrose, did not form indol; type E did not ferment dextrose or lactose, did not coagulate milk, did not form indol. The only characters which these "colon bacilli" exhibited in common were the "classical growth" upon potato, the possession of less than eight cilia, and the power of active development upon certain media upon which the typhoid bacillus did not grow. A more careful and significant piece of work on the same line was published by Poujol in the succeeding year. This author reported¹⁷ the isolation of *B. coli* from twenty-two out of thirty-four waters studied by him in relation to their use as public supplies. The waters were from various sources, springs, wells, and rivers, but all were of fair quality and many quite free from any possibility of contamination. Samples of one hundred cubic centimeters were used for analysis; in the only case in which a smaller amount was also tested, broth inoculated with ten drops of the water and placed at 45° C. remained sterile. The author concluded that "fecal contamination can only exceptionally be invoked to explain the presence of *B. coli* in water. As

the bacteria of the subterranean water are contributed to it from the surface of the earth by the water which filters downward, I am rather inclined to believe in a general diffusion of *B. coli* either on the surface of the earth, where it might be deposited with the dust of the air or in the superficial layers of the earth which may form one of its normal habitats." Therefore the author considered that caution should be exercised in condemning a water on account of the presence of *B. coli*, except, as he added, "for those cases where it exists in considerable quantity."

Certain Italian observers appear to have come to even less conservative conclusions. Abba¹⁸ in 1895 found colon bacilli constantly present in certain unpolluted waters near Turin. Moroni in 1898¹⁹ and 1899²⁰ reported the examination of numerous deep and shallow wells and unpolluted springs about Parma, as well as of the public water supply of the city, for the colon bacillus and concluded that that organism was a water form and had no sanitary significance. The characters used for the identification of the species in this case were fairly exhaustive, but both Abba and Moroni used liter samples for analysis.

Levy and Bruns²¹ gave a new turn to the discussion by emphasizing the importance of animal inoculation, already suggested by Blachstein²² and others. They claimed that the existence of numerous para-colon and para-typhoid organisms, in air, in dust, and in unpolluted water, made it impossible to decide by ordinary bacteriological methods whether true colon bacilli were present in water or not. In no case, however, did representatives of the colon group isolated by them from water kill a guinea-pig, even when one or two centimeters were injected intra-peritoneally; and the authors therefore considered pathogenicity as an attribute belonging only to the true *B. coli* of the intestine. This paper aroused Professor Kruse's pupil, Weissenfeld, to a publication, in which the position of the Bonn school was carried to an extreme. Weissenfeld reported²³ the analysis of thirty samples of water supposedly pure, and of twenty-six samples considered to be contaminated. In each case a

single centimeter sample was first incubated in Parietti broth, and if no growth occurred, larger samples of half a liter or a liter were examined. Colon bacilli were found in all the samples examined; and the pathogenicity varied independently of the source of the water. The author concluded that "the so-called *Bacterium coli* may be found in waters from any source, good or bad, if only a sufficiently large quantity of the water be taken for analysis." In the first place it should be noted that the characters used by this investigator for defining the "so-called *Bacterium coli*" were absolutely inadequate. He classed under that head all bacilli of medium size, which formed grape-vine-leaf colonies on gelatin and gas in sugar-agar, which were more or less motile, or rarely non-motile, and which were decolorized by the Gram method. As regards coagulation of milk and formation of indol, "the bacteria isolated differed." In the second place it is difficult to see how the author could possibly have believed that his experiments proved the isolation of the colon bacillus to be "useless as an aid in the sanitary examination of water," as the title of the paper runs. Even his own work furnishes strong evidence to the contrary. In twenty-four of the twenty-six samples from bad sources, he isolated his imperfectly defined colon bacilli from one cubic centimeter of the water, while in only eight of the thirty samples of good waters could he find such organisms in that quantity.

The most striking contribution to this side of the question was communicated to the American Society of Bacteriologists by Prescott²⁴ from the same laboratory in which our own work has been carried on. He examined forty-seven cultures of lactic acid bacteria, some obtained by washing various cereals and some in technical use for producing the lactic fermentation, and after a thorough series of comparative tests, found that twenty-five of the cultures gave the typical reactions of *B. coli*. The author concluded that "too much reliance must not be placed upon the so-called colon test of potable waters."

All these observers have ignored what seems to us to be the crucial point in the sanitary application of the colon test—

the approximate number of the organisms present in water. It may well be that colon bacilli originally derived from the intestine occur in rivers and grain fields far from any source of immediate pollution. As Dr. Smith said in 1896,²⁶ "It is true that they are widely distributed in nature, mainly because fecal discharges of human beings and animals are a common thing on the soil." It may be, on the other hand, as Prescott's work seems to suggest, that the organism is originally a saprophyte which adapts itself to the intestine, as a secondary home. In any event, the colon bacillus appears to find in the intestine of the higher vertebrates an environment better suited to its growth and multiplication than any other which occurs in nature. It is extremely probable that a vast majority of the colon bacilli in the world at any one time are to be found in such a habitat. It is almost certain that the only way in which large numbers of these organisms gain access to natural waters is by pollution with the domestic, industrial, and agricultural wastes of human life.

The recognition of the necessity for a quantitative estimation of colon bacilli in water we owe mainly to Dr. Smith, who in 1892²⁶ outlined a plan for such a study to be made by the New York Board of Health on the Mohawk and Hudson rivers. Burri²⁷ in 1895 pointed out that the use of so large a sample as a liter for examination would lead to the condemnation of many good waters. Freudenreich²⁸ at the same time pointed out the necessity for taking into account the *number* of colon bacilli present. He recorded the isolation of the organism from unpolluted wells, when as large a quantity of water as one hundred cubic centimeters was used, and concluded that it was entirely absent only from waters of great purity and present in large numbers only in cases of high pollution. This author also quoted Miquel as having found colon bacilli in almost every sample of drinking water, if only a sufficient portion were taken for analysis, but gave no reference. Smith in 1895²⁶ stated that he considered "the inoculation of a series of tubes of more service than the addition of a large quantity of water to bouillon in a single

flask," and in 1896 he put the case still more strongly, as follows: ²⁸ "The mere presence of the colon bacillus does not necessarily mean pollution with human excrement, nor does it mean that the water should not be used. What we do wish to find out is *how many* colon bacilli are in a given quantity of water."

The practical results of the application of the colon test from this standpoint have always proved most instructive. As originally outlined by Dr. Smith, it consisted in the inoculation of a series of dextrose tubes with small portions of water, tenths or hundredths of the cubic centimeter. It was first used by Brown ²⁹ in 1892, for the New York State Board of Health; and its results showed from twenty-two to ninety-two fecal bacteria per cubic centimeter in the water of the Hudson river at the Albany intake, and from three to forty-nine at various points in the Mohawk river between Amsterdam and Schenectady. In some previous work at St. Louis, the colon bacilli in the Mississippi river were found to vary from three to seven per cubic centimeter; and the author stated that their numbers, "in water supplies from sources considered on inspection to be unpolluted, vary from none to five." This last clause evidently referred to river supplies of a character which would hardly be considered satisfactory to-day.

Hammerl in 1897 ³⁰ used the presence of *Bacillus coli* as a criterion of self-purification in the river Mur. He considered, in spite of the position taken by Kruse, that when a water contained large numbers of colon bacilli, as well as an excess of bacteria in general, it might be considered to be contaminated by human or animal excrement. As, however, the organism would naturally be present in large quantities of such a water as that of the Mur, he used no enrichment process, but made plate cultures from the cubic centimeter and from one-half the cubic centimeter; he defined the *B. coli* as a small bacillus, non-motile or but feebly motile, growing rapidly at 37° C., coagulating milk and forming gas in sugar media. In general Hammerl failed to find colon bacilli in the river by this method, except immediately below the various towns situated upon

it; at these points of pollution he discovered a few colon colonies upon his plates, not more than four to six per cubic centimeter of the water. He concluded that "the *Bacterium coli*, even when it is added to a stream in great numbers, under certain circumstances disappears very rapidly, so that it can no longer be detected in the examination of small portions of the water." It should be noted that Hammerl's method was much less delicate than the use of the dextrose tube for preliminary incubation.

The most important work of the last few years in sanitary water analysis is that which has been carried out by the bacteriologists of the Local Government Board of England, and published in the annual reports of the Medical Officers of Health. These really brilliant investigations have received much less attention than they deserve both in this country and on the continent. In the first place Klein and Houston made some most striking comparisons of the delicacy of the bacteriological and the chemical tests, by examining mixtures of sewage and water, prepared in various dilutions.³¹ While ordinary chemical methods failed to detect one part in one thousand of the sewage, the presence of *B. coli* and *B. sporogenes* indicated pollution to the extent of one-tenth to one-hundredth of that quantity.

The second line of work, carried out at the English Health Office, this time by Dr. Houston, was the examination of soils from various sources to see whether the microbes considered to be characteristic of sewage could gain access to water from surface washings free from human contamination.³² In the three papers published on this subject the examination of forty-six soils was recorded. In only ten of the samples was *B. coli* found, and of these ten, nine were obviously polluted, being derived from sewage fields, freshly manured land, or the mud banks of sewage polluted rivers. The author finally concluded that "virgin soils do not contain spores of *B. enteritidis sporogenes*, *B. coli* (or closely allied forms), or streptococci, or at most they contain these microbes only in small number. . . . Soils polluted with animal organic matter contain spores of *B. enteritidis sporogenes*.

genes in great abundance, and also *B. coli* and streptococci if the contamination is of recent sort." In 1899-1900 Klein and Houston reported³⁸ the bacterial analysis of certain food stuffs, their results being interesting in relation to those obtained by Prescott in this country. Typical colon bacilli were found by the English observers in three out of twenty-four samples of wheat and oats obtained from a wholesale house, and colon-like organisms, most of which slowly liquefied gelatin, in seven other cases. Rice, flour, and oatmeal bought at two different retail shops gave *B. coli* on all three cereals in one case and on none in the other.

The third service rendered by Klein and Houston to the cause of sanitary water-analysis was the discovery of two new and apparently characteristic sewage organisms to confirm and supplement evidence furnished by the *B. coli*. These forms are the *B. sporogenes* of Klein³⁴ and the sewage streptococci of Houston.³⁶ The latter investigator declared in 1898-9 that the streptococci "are organisms readily demonstrable in waters recently polluted and seemingly altogether absent from waters above suspicion of contamination. . . . Search for them should . . . constitute an important part of the bacterioscopic analysis of potable waters." In the next year the study of these new forms was carried further. In the water of six rivers, recently extensively sewage polluted, streptococci were found in from one-tenth to one ten-thousandth of a cubic centimeter of the water examined, although in some cases the chemical analysis did not clearly indicate dangerous pollution. On the other hand, eight rivers, polluted but not recently and extensively polluted, showed no streptococci in one-tenth of a cubic centimeter although the chemical and the ordinary bacteriological tests gave results which would condemn the waters. Houston's final conclusion as to the significance of these organisms was as follows: "As a matter of actual observation, the *relative abundance* of *B. coli* in pure and impure substances is so amazingly different as to lead us to suspect that not only does *B. coli* not flourish in nature under ordinary conditions, but that it tends to even lose its vitality and die." "In brief,

I am strongly of opinion that the presence of *B. coli* in any number, whether in soil or in water, implies *recent* pollution of animal sort. But is there any microbe (or class of microbes) which by its presence implies animal pollution of *extremely recent*, and therefore *specially dangerous* kind? The streptococci, in my judgment, are to be thought of in this sense."

Other English bacteriologists have, in general, simply confirmed and extended these results. Thus Pakes³⁶ concluded from the examination of "about three hundred different samples of water," no particulars being published, that water from a deep well should not contain *B. coli* at all, but that water from other sources need not be condemned unless the organism was found in twenty cubic centimeters or less. When colon bacilli were found only in greater quantities than one hundred cubic centimeters the water might be considered as probably safe.

Finally Horrocks³⁷ in 1901, after a general review of English practice, concluded that "when a water supply has been *recently* polluted with sewage, even in a dilution of one in one hundred thousand, it is quite easy to isolate the *B. coli* from one cubic centimeter of the water." "I would say that a water which contained *B. coli* so sparingly that two hundred cubic centimeters required to be tested in order to find it, had probably been polluted with sewage, but the contamination was not of recent date."

In the United States the colon test has been extensively applied during the last few years, to certain polluted river waters, in particular with the view of measuring the purification attained by sand filtration and that naturally occurring during the flow of a stream. A fairly good idea has thus been obtained of the numerical distribution of the *B. coli* in the larger rivers. Fuller, for example, in 1899³⁸ recorded the presence of colon bacilli in sixty per cent of the one cubic centimeter samples taken from the Ohio at Cincinnati. When this water was passed through either slow sand or mechanical filters, the effluent gave positive results about one-half the time in samples of fifty cubic centimeters.

The last three reports of the Massachusetts State Board of

Health³⁹ contain reports of the abundance of *B. coli* in a more highly polluted stream, the Merrimac at Lawrence. In 1898 the number of organisms found by making litmus-lactose-agar plates directly and inspecting the colonies, varied from twenty per cubic centimeter in May and June to ninety-two per cubic centimeter in August and September, the average for the year being forty-seven. Of one hundred and seventeen samples of the water which had passed through the city filter, only nine showed the organism in a single colony.

In 1899 the study was considerably extended. The average number of colon bacilli in the river at the intake of the filter was again forty-seven, and in only one sample out of one hundred and eighty was it absent; below the city at the Lawrence Experiment Station the additional pollution raised the average number to one hundred and three. The effluent as it came directly from the filter showed *B. coli* in twenty-four per cent of the cubic centimeters examined, but at the outlet of the reservoir, the proportion had fallen to seven per cent and at the Experiment Station, after passage through the distribution pipes, to only four per cent. The results obtained in the next year, 1900, were practically the same, but parallel tests were made in a larger volume of water by incubating with the addition of phenol-broth. The results of these comparative tests may be tabulated as follows:

Percentage of Samples of Water containing B. coli.

	Effluent of Filter.	Outlet of Reservoir.	Tap, City Hall.	Tap, Experiment Station.
In 1 cc.	18.14	8.57	4.07	1.87
In 100 cc.	38.12	23.30	15.54	15.54

It appeared that the use of the larger volume of water gave very little additional information, and indeed the real difference between the waters examined is rather obscured by the use of the large samples.

In 1900 Clark and Gage⁴⁰ reported some specially instructive observations made when certain of the underdrains

of the Lawrence municipal water filter were relaid in the autumn of 1898. In doing this work the sand on some of the beds was seriously disturbed; and in December, after the work was completed, *B. coli* was found in one cubic centimeter of the filtered effluent in seventy-two per cent of the samples examined. In January and February the organisms were found in fifty-four per cent and sixty-two per cent of the samples, respectively, while in March the number fell to a normal value of eight per cent. Corresponding to this excess of *B. coli* in the city water, there were twelve cases of typhoid fever in December, fifty-nine cases in January, twelve in February, and nine in March, all during the early part of the month. The authors conclude that "when filtering a river water as polluted as that of the Merrimac, it is safe to assume that when *B. coli* is found only infrequently in one cubic centimeter of the effluent the typhoid germs, necessarily fewer in number and more easily removed by the filter, have been eliminated from the water."

Another interesting contribution to this question was made by the Massachusetts State Board of Health, in connection with the examination of the spring waters bottled for sale in the State, which was carried out in 1900. Ninety-nine springs were included in this study; and in almost every instance four samples were examined, two taken directly from the spring by the engineers of the board and two from the bottles as delivered for sale to the public. In the water of one spring *B. coli* was found twice, once in a sample from the spring and once in the bottled sample. This spring was situated in woodland, but was unprotected from surface drainage, and the method of filling bottles subjected it to possible contamination. In five other cases *B. coli* was found once in the sample from the spring; all were subject to pollution from dwellings or cultivated fields, and four of the five were shown to be highly contaminated, chemically. In seven other cases *B. coli* was found in the bottled samples alone; three of these sources were of high purity, but the bottling process furnished opportunity for human contamination.

Probably the most elaborate application of the colon test

which has ever been attempted was made by Jordan in his recent examinations of the fate of the Chicago sewage in the Desplaines and Illinois rivers. It is interesting to note that at one time Professor Jordan was himself somewhat sceptical as to the value of the colon test, for he stated in 1890⁴² that he had found, "in spring water which was beyond any suspicion of contamination, bacteria which in form, size, growth on gelatin, potato, etc., were indistinguishable from *B. coli* commune." In his recent studies of self purification⁴³ the analyses were made quantitative by the examination of numerous measured samples, fractions of the cubic centimeter; and the method employed was sometimes the direct inoculation of dextrose broth fermentation tubes and sometimes the incubation of the water in phenol-broth, with the subsequent making of litmus-lactose-agar plates. The cultures isolated on these plates were tested as to their behavior in dextrose-broth, peptone solution, milk, and gelatin; of the dextrose tubes made directly from the water all were considered positive which gave more than twenty per cent gas in the closed arm, with an appreciable excess of hydrogen. The results were very significant. In fresh sewage a positive result was obtained about one-third of the time in one one-hundred-thousandth of a cubic centimeter and almost constantly in one ten-thousandth of a cubic centimeter. The Illinois and Michigan canal proved almost as bad, giving positive results on seven days out of twenty-eight in dilution of one in one hundred thousand and on twenty-eight days out of thirty-two in a dilution of one in ten thousand. At Morris, twenty-seven miles below Lockport, where the canal enters the bed of the Desplaines river (an insignificant stream), and nine miles below the entrance of the Kankakee, the principal diluting factor, the numbers were so reduced that positive results were obtained only on eleven days out of twenty in one-thousandth of a cubic centimeter, on twenty days out of thirty in one-hundredth of a cubic centimeter, and on twenty days out of twenty-three in one-tenth of a cubic centimeter. At Averyville, one hundred and fifty-nine miles below Chicago, the effect of the sewage had practi-

cally disappeared, colon bacilli being isolated on only four days out of twenty-seven in one-tenth of a cubic centimeter, and on thirteen days out of thirty-one in one cubic centimeter. A comparison with certain neighboring rivers showed this to be about the normal value for waters of that character, as the following table extracted from Professor Jordan's paper will show:

Source of Sample.	.1 cc.		1 cc.	
	No. days water examined.	No. days B. coli found.	No. days water examined.	No. days B. coli found.
Illinois river, Averyville,	27	4	31	13
Mississippi river, Grafton,	34	10	35	23
Fox river,	22	2	23	6
Sangamon river,	25	14	27	21
Missouri river,	32	13	31	21

These results harmonize rather closely with those previously recorded by Brown and Fuller and indicate that in the larger rivers where the proportionate pollution is not extreme, colon bacilli may be isolated in about half the one cubic centimeter samples examined. Such rivers are of course inadmissible as sources of water supply, according to modern sanitary standards, unless subjected to preliminary treatment.

II. METHOD OF ANALYSIS PURSUED IN THIS INVESTIGATION.

In spite of the wide practical application of the colon test, indicated by the publications cited above, it seemed to us desirable that more experimental work should be done upon the distribution of B. coli in natural waters. In the first place the available data bearing on the presence of this organism in unpolluted waters, in such abundance as to be commonly found in small samples, are really very meager. In the second place, the papers of Kruse and Poujol, and, in

particular, of Weissenfeld, appear to have produced an impression out of all proportion to their real significance. It was for these reasons that we undertook the examination of a considerable series of normal waters, using a small as well as a large portion in each case, and applying a sufficient number of bio-chemical tests to identify the organisms isolated with some degree of accuracy.

The samples of water to be tested were collected in one hundred cubic centimeter sterilized bottles and reached the laboratory in every case three or four hours after collection. One cubic centimeter was added to a tube of dextrose-broth; and about ten cubic centimeters of phenol-dextrose-broth (broth containing ten per cent dextrose, five per cent peptone, and one-fourth per cent phenol) was added to the main sample in the original bottle. The dextrose tube and the bottle were then incubated at 37° C. for twenty-four hours. If at the end of that time the dextrose tube showed no growth or growth but no gas, the test in one cubic centimeter was considered negative. If gas was present in any amount a litmus-lactose-agar plate was made and placed in the incubator for twenty-four hours more. From the one hundred cubic centimeter bottle, in every case where any growth occurred, a cubic centimeter was taken to inoculate a dextrose tube which, if positive after twenty-four hours, was also used for the inoculation of a litmus-lactose-agar plate. It has been suggested that the formation of a persistent froth on shaking a hundred cubic centimeter bottle after incubation as described would serve to indicate whether or not dextrose fermenting organisms were present. We were unable to find any constant relation between the appearance of the bottle after shaking and the gas formation in the dextrose tube inoculated from it.

If only blue colonies appeared on either of the litmus-lactose-agar plates after twenty-four hours, that part of the test was considered negative. The weak point in any isolation, based on preliminary incubation, is the possibility that colon bacilli, originally present in the dextrose tube, may be overpowered by some other form, and so may not appear on the

lactose plate. In fact, we have evidence that this sometimes occurs, as will be shown in the discussion of the results obtained in the analysis of polluted river waters. It is not probable, however, that fairly pure waters contain organisms capable of development at the body temperature sufficiently rapid to check the growth of *B. coli*.

If red colonies of any sort were found on the lactose plate, three of them were fished and isolated on agar streaks. Usually only one type of colony appeared on a plate; if several were noticed, one streak was made from each sort. The pure cultures thus obtained were then examined by subcultures in dextrose broth, milk, nitrate solution, peptone solution, and gelatin. Inspection of the streaks at once showed two types of growth, the abundant, first translucent, later whitish and cheesy growth, covering nearly the whole surface of the agar, characteristic of *B. coli* and its allies, and a very faint growth, either confined strictly to the streak or made up of faint, isolated colonies, dotted here and there over the surface. Only those organisms of the first type were considered to be colon bacilli which gave the following reactions: the fermentation of dextrose broth with the production of gas in twenty-four hours; the fermentation of lactose in the litmus-lactose-agar plate, with distinct reddening, in twenty-four hours; the production in milk of acid sufficient to cause it to coagulate on heating after twenty-four hours; the production of nitrites from nitrates in twenty-four hours; the production of indol in peptone solution in three days — all the above at 37° C.; and the formation of the typical gelatin stab-growth of *B. coli*, with no liquefaction of the gelatin in seven days. According to the valuable schematic analysis of the colon group prepared by Ford in 1900⁴⁴ these tests would correspond to the strict *B. coli* of Escherich. In his later paper⁴⁵ the same author makes only two groups of the acid-forming intestinal organisms which do not liquefy gelatin, — the colon group including motile forms which produce indol and the *lactis aerogenes* group containing non-motile forms which do not produce indol. In each group are three types, the first fermenting dextrose, lactose, and saccharose, the second

dextrose and lactose, the third, dextrose alone. Our standard, as above outlined, would include the first two types of the colon group.

The organisms which gave colon-like streaks, but failed to conform to some of the later tests, proved in a few instances to be liquefying forms belonging to the *B. cloacæ* group. The rest resembled the colon bacillus, lacking only one or two characteristics, usually the power to reduce nitrates or to form indol. These we have classed as para-colon organisms. The idea naturally suggests itself that they may be true colon bacilli which have lost certain functions by sojourn in an unfavorable environment.

The organisms which gave the faint dotted growth on the agar streak produced acid but no gas in the dextrose tube, and coagulated milk but formed neither nitrites nor indol. A few of them slowly liquefied gelatin, but most did not. All grew best under anaerobic conditions and but feebly in media not containing sugar. When these forms appeared in many of the samples of polluted waters they were examined in more detail and found to belong to the groups of streptococci and staphylococci, which Houston considered so characteristic of sewage.

As far as we are aware, these organisms were first reported in this country in a preliminary communication made by ourselves.⁴⁶ They were originally isolated by us in a study of the bacteria occurring on the hands, chiefly of students and school children; they have since been found in fresh feces, in Boston sewage, and, by Mr. D. M. Belcher, a student working in the Institute laboratories, in a study of the bacteria in a septic tank. As will be seen later, our work appears to confirm the conclusion of Houston that the streptococci and staphylococci are of great significance in the sanitary examination of water.

III. EXAMINATION OF ONE HUNDRED AND FIFTY-SEVEN SAMPLES OF WATER FROM PRESUMABLY UNPOLLUTED SOURCES.

(The waters analyzed in this investigation were all obtained from towns and cities in the eastern part of New England; and, with the exception of those from Taunton, Mass., and Newport, R.I., all from points within thirty miles of Boston. The various sources are grouped under class headings in the first column of Table I.)

In the collection of the samples the attempt was made to cover as wide a range of waters as possible, without including any which were subject to pollution. The only exceptions to this last clause were the samples from the Sudbury river, a stream which is polluted, but only at a considerable distance from the points where the samples were taken, and the various brooks. These latter were not all inspected along their course and some of them, in particular at Lincoln and at Framingham, may have been contaminated above the places where samples were taken.

The samples from the public supplies of Boston, Brookline, Needham, Lynn, and Newport were obtained from taps (in the first four cases at weekly intervals), during the autumn of 1900. Of the Cambridge samples, most were collected in the same way, one being taken, however, directly from Fresh Pond. The Taunton samples were collected in December, 1900, some from taps in the city and at the Lakeville pumping station, and the rest from selected points in Long Pond, Assawompsett Lake, and Elder's Pond. The Braintree samples were obtained from the pump well at the pumping station in October, 1901. It should be noted that the supplies of Boston, Lynn, Taunton, Cambridge, and Newport are surface waters, and those of Brookline, Needham, and Braintree are obtained from the ground.

The ponds examined included Walden Pond, Concord, the old East Lexington Reservoir, two of the small ponds in the Boston Public Garden, and others in Dedham, Framingham, Sharon, Melrose, and West Roxbury, — eleven different sources in all. The five shallow well samples were obtained from three wells, one in Dedham and two in Framingham, ap-

parently free from pollution. The deep wells, with one exception, belonged to various breweries in the Jamaica Plain district of Boston; two samples were taken in most cases, nine different wells being represented. The depth of these wells was said to vary from one hundred and sixty feet to seven hundred and fifty feet; the region in which they are situated is thickly settled. The springs studied, fourteen in number, were in the towns of Dedham, West Roxbury, Boston, Medford, Sharon, and Framingham. The nineteen brook samples were obtained from sixteen different brooks situated in the towns mentioned, and in one case in Lincoln. The twenty-five samples under the heading "pools of rain and melted snow" were collected from woodlands and fields in Framingham, Melrose, Boston, Medford, Dedham, Sharon, and West Roxbury; many were very dirty and all contained decomposing vegetable matter and washings from the surface of the ground. Most of these natural sources were examined during the spring of 1902, from February to April; a few, during the autumn of 1900, from October to December.

The general results of the analyses are shown on the appended tables, Table I. exhibiting the results of the single centimeter examinations, and Table II. of those carried out with the larger samples. The differences in the total number of samples in the two tables is due to the loss of several cultures by accidents and to the fact that in a few cases the examination in one hundred cubic centimeters was omitted.

Table I. shows that of one hundred and fifty-seven dextrose tubes inoculated with one cubic centimeter of the water examined, one hundred and seventeen showed no gas production in twenty-four hours at 37°. The plates made from the forty tubes which did contain gas gave in twenty-seven cases only blue colonies. From the remaining thirteen plates sub-cultures were made, and from five typical colon bacilli, as defined above, were isolated. The origin of these five samples was as follows: Boston water from a tap in the laboratory, Cambridge water from Fresh Pond, brooks in Lincoln and Framingham, and a pool of melted snow and ice in a meadow in Jamaica Plain. The organisms present in the other eight

samples have been roughly classified in the table under three heads, according to the results of the five tests applied (growth in dextrose broth, milk, nitrate solution, peptone solution, gelatin), although no attempt has been made to work them out further. As para-colon organisms we have classed those which produced gas in dextrose broth, and gave typical growth in gelatin, but failed, either to reduce nitrates or to form indol, or to form sufficient acid to coagulate milk. Bacteria of this sort were also isolated from some of the plates on which true colon bacilli were formed. In the last column are included organisms which coagulated milk, grew feebly in gelatin, produced no gas, and did not reduce nitrates or form indol. These reactions, as noted above, are typical of the streptococci and staphylococci.

Of the one hundred and fifty-three waters of which one hundred cubic centimeter samples were examined, seventy-seven gave no gas in the dextrose tube, and of the other seventy-six, forty-five produced no red colonies on the agar plate. Thirty-one showed fermentation in lactose-agar and were studied further. In eleven cases *B. coli* was found: from the Boston sample and the Framingham and Lincoln brooks above mentioned, from a Cambridge tap sample, from a spring on a wooded crest in Medford, from two other brooks in Framingham, from a point in the Taunton supply close to a roadway, from a pool of melted snow in a Framingham meadow, and from two of the brewery wells. It may be noted that the Medford and Framingham samples were collected in early spring after heavy thaws, when contamination from long distances might easily reach any surface water. Organisms closely related to *B. coli*, as described above, were found in five cases; in two of the samples of spring water from the same source, both in the large and small samples, a peculiar form occurred which gave all the colon reactions strongly except that it failed to form indol. In five samples a form was found which resembled *B. coli* in most respects, but liquefied gelatin; this we have assigned to the "*B. cloacæ*" group. In ten cases the organisms isolated gave the reactions which we have described as associated with the group of the streptococci and staphylococci.

TABLE I.

Unpolluted Waters. Analyses of 1 cc. Samples.

SOURCE OF SAMPLES.	Number of samples.	Dextrose tube positive.	Lactose plate positive.	Colon group.	Paracolon group.	B. cloacæ group.	Streptococcus group.
Taunton supply (ponds and taps)	12	0					
Boston supply (taps)	9	3	2	1	1
Cambridge supply (pond and tap)	9	6	2	1	1		
Braintree supply (pump well)	8	0					
Brookline supply (tap)	7	0					
Needham supply (tap)	5	0					
Lynn supply (tap)	3	2	0				
Newport (R.I.) supply (tap)	1	0					
Bottled spring waters	7	3	2	2		
Rain water	2	0					
Sudbury river	2	1	0				
Sea water (Cotuit harbor) ..	2	0					
Ponds	12	8	1	1
Shallow wells	5	1	0				
Driven wells	17	1	1	1		
Springs	14	1	0				
Rain pools and pools of melted snow	23	7	2	1	1		
Brooks	19	7	3	2	1
Totals	157	40	13	5	5	3

TABLE II.

Unpolluted Waters. Analyses of 100 cc. Samples.

SOURCE OF SAMPLES.	Number of samples.	Dextrose tube positive.	Lactose plate positive.	Colon group.	Paracolon group.	B. cloacæ group.	Streptococcus group.
Taunton supply (ponds and taps)	12	3	3	1	1	1
Boston supply (taps)	8	8	4	1	1	2
Cambridge supply (pond and tap)	8	7	1	1			
Braintree supply (pump well)	4	3	1	1		
Brookline supply (tap)	6	3	0				
Needham supply (tap)	5	0					
Lynn supply (tap)	3	2	1	1
Newport (R.I.) supply (tap)	1	0					
Bottled spring waters	7	4	2	2		
Rain water	2	0					
Sudbury river	2	1	1	1
Sea water (Cotuit harbor) ..	2	1	0				
Ponds	14	6	4	2	2
Shallow wells	4	1	1	1
Driven wells	17	3	2	2			
Springs	15	7	2	1	1
Rain pools and pools of melted snow	25	15	3	1	1	1
Brooks	18	12	6	4	2	
Totals	153	76	31	11	5	5	10

IV. EXAMINATION OF FIFTY SAMPLES OF WATER FROM OBVIOUSLY POLLUTED SOURCES.

In order to obtain a basis for comparison, we examined fifty samples of water from obviously polluted sources by the same method outlined above. These samples were collected in lots of nine or ten each from points several hundred feet apart along the banks of the following streams, all in the eastern part of Massachusetts: the Charles river at Boston, off the water-front behind Beacon street, the Neponset river at Hyde Park, the Mystic river between Charlestown and Everett, and the North river at Salem. Except the Neponset river these are tidal streams, and in every case the samples were collected on a falling tide. All these rivers receive considerable quantities of sewage, while the Neponset river and the North river are very highly polluted with manufacturing waste as well.

With the forty-eight river samples are included two of a well water, from a shallow well at Newport, grossly contaminated and probably responsible for an epidemic of typhoid fever which occurred during the fall of 1900.

Of the dextrose tubes inoculated with one cubic centimeter samples of these polluted waters, every one showed gas formation after twenty-four hours; and on all the plates made from these tubes red colonies were produced. Of the pure cultures isolated from these plates, however, only half gave gas in the dextrose tube. It was, therefore, evident that some form originally present, which produced gas in the first dextrose tube, must have been overgrown during the latter part of the twenty-four-hour period of incubation and replaced by an organism which fermented the sugar with the formation of acid, but no gas. The form which thus finally appeared in one-half of the total samples examined gave the reactions associated with the group of the streptococci and staphylococci. We have headed the column in the tables, "Streptococcus Group," as Houston laid principal emphasis upon this form. In most of the stained preparations made from agar streaks of the organisms, short chains and small irregular groups of cocci were observed; and in a few cases only

isolated cells and diplococci were apparent. All gave the characteristic growths in agar and in dextrose broth and milk described above; all failed to form indol and reduce nitrates; a few liquefied gelatin, but most did not.

In eighteen samples typical colon bacilli were found, and in six cases related forms which failed to form nitrites or indol. It is reasonable to assume that the organisms which produced gas in the preliminary dextrose tube in the other twenty-five samples were of the same general character. It seems probable, then, that colon bacilli were present in all the samples and that in half of them they were overgrown by the representatives of the streptococcus group.

Similar phenomena are shown in a still more striking manner on Table IV. where the results of the analyses of the large samples are tabulated. It will be seen that of the forty-eight dextrose tubes, inoculated from the one hundred cubic centimeter bottles, seventeen failed to give gas in the first dextrose tube. Now, we know from the examination of the one cubic centimeter samples of these same waters that dextrose fermenting organisms were originally present in every case. They must have been suppressed, then, in these seventeen samples during the incubation with phenol broth; and it is probable that the form which became dominant was the streptococcus again. Of the twenty-six samples which showed red colonies on the agar plate, only four contained *B. coli*, and the rest streptococci and staphylococci. Thus the dextrose fermenting organisms, which the immediate inoculation of the dextrose tube showed to be present in all these forty-eight samples, disappeared in forty-four cases during the two incubations of twenty-four hours each.

TABLE III.

Polluted Waters.—Analyses of 1 cc. Samples.

SOURCE OF SAMPLE.	Date of collection.	Number of samples.	Dextrose tube positive.	Lactose plate positive.	Colon group.	Paracolon group.	Streptococcus group.	B. cloacæ group.
Charles river..	Mar. 17, 1902.	9	9	9	1	8	
Neponset river.	Mar. 24, 1902.	9	9	9	7	1	1
Charles river .	Mar. 31, 1902.	10	10	10	1	9	
Mystic river ..	Apr. 7, 1902 .	10	10	10	4	3	3	
North river...	Apr. 14, 1902.	10	10	10	4	1	5	
Newport well .	Oct. 12, 1900.	2	2	2	1	1		
Totals	50	50	50	18	6	25	1

TABLE IV.

Polluted Waters. Analyses of 100 cc. Samples.

SOURCE OF SAMPLE.	Date of collection.	Number of samples.	Dextrose tube positive.	Lactose plate positive.	Colon group.	Streptococcus group.
Charles river.....	Mar. 17, 1902	9	6	4	1	3
Neponset river	Mar. 24, 1902	9	9	9	2	7
Charles river.....	Mar. 31, 1902	10	6	4	4
Mystic river	April 7, 1902	10	6	5	1	4
North river	April 14, 1902	10	4	4	4
Totals	48	31	26	4	22

V. CONCLUSIONS.

As to methods of analysis :

1. It appears evident that the use of large samples in applying the colon test to the sanitary analysis of drinking water is not advantageous. In comparing the results of the tests in one cubic centimeter and in one hundred cubic centimeters, it will be noted that the proportion of lactose fermenting organisms and of colon bacilli in the unpolluted waters, was more than doubled in the latter; thus waters of good quality are more likely to be condemned by the use of large samples. On the other hand, in the polluted waters a considerable proportion of the colon bacilli originally present were lost during the incubation of the large samples, so that waters of bad quality actually appeared to better advantage by the use of one hundred cubic centimeters with preliminary incubation in phenol broth.

2. With polluted waters, at least, it is evident that even the incubation of a single cubic centimeter for twenty-four hours in dextrose broth may lead to the suppression of colon bacilli. It is possible, too, that in some of the twenty-seven samples of unpolluted waters, in which the dextrose tube showed gas and the lactose plate was negative, colon bacilli may have been overgrown. The idea suggests itself that in the routine sanitary examination the use of the dextrose tube alone with simple determination of the gas formula, or the direct plating of the water in litmus-lactose-agar, without preliminary incubation, might give more useful, practical results in some cases than the usual procedure. According to the work of Irons ⁴⁷ the direct inoculation of the lactose plate is not successful with polluted waters. On the other hand, he concluded that "when the dextrose tube method yields approximately thirty-three per cent of carbon dioxide, *B. coli* is almost invariably present." It is very desirable that more comparative work should be done on the three principal tests for fecal bacteria, the use of the dextrose tube alone, with record only of the gas formula, the use of the litmus-lactose-plate, without preliminary treatment, and the use of the dextrose tube, followed by the litmus-lac-

tose-agar plate. If the view of the English bacteriologists as to the importance of the streptococcus group be accepted, the direct use of the litmus-lactose-agar plate gains in importance, since these forms produce acidity, but no gas.

3. It is manifestly unsafe to assume, as some have done, that red colonies on the litmus-lactose-agar plate indicate the presence of organisms of the colon group. Besides *B. coli* and closely related forms, we have found bacilli related to *B. cloacæ* in a few instances, and in very many cases streptococci and staphylococci. Subcultures should then be made from the red colonies on the lactose-agar plate whenever it is desired to form any idea as to what groups are represented. In most cases the appearance of the ordinary agar streak serves at once to distinguish the group of the streptococci and staphylococci, which produce only a faint growth, either confined to the streak, or dotted over the surface in the form of small, circular, transparent colonies.

As to the interpretation of results:

4. Comparison of the results of the analyses made by using one cubic centimeter samples shows a striking difference in the distribution of the sugar fermenting organisms in general. Under the conditions of the experiment (incubation in dextrose broth for twenty-four hours at 37° C.) all of the fifty samples of polluted water showed gas production against only about one-quarter of the samples of unpolluted water (forty out of one hundred and fifty-seven). Furthermore, of the litmus-lactose-plates made from these dextrose tubes, the entire fifty showed red colonies in the first class of waters, while only about eight per cent of the plates were reddened in the second class (thirteen out of one hundred and fifty-seven). It seems, then, that bacteria capable of growing at the body temperature and fermenting dextrose and lactose are only infrequently present in unpolluted waters.

5. These experiments show that by the method employed, *B. coli* is very rarely found in one cubic centimeter samples of unpolluted waters. In one hundred and fifty-seven samples typical colon bacilli only appeared five times, and para-

colon organisms five times more. This confirms the observations of Smith and the English bacteriologists as to the parallelism between the number of colon bacilli present in a water and the extent of its contamination. As has been stated by one of us, "When the colon bacillus, as defined by the tests noted above, is found in such abundance as to be isolated in a large proportion of cases from one cubic centimeter of water, it is reasonable proof of the presence of serious pollution." ⁴⁸

6. The group of the streptococci and the staphylococci appears, as in the observations made by Houston and Horrocks, to be associated with sewage pollution when present in any numbers. While these organisms occurred in only three, out of one hundred and fifty-seven, one cubic centimeter samples of unpolluted waters, they were isolated from twenty-five out of fifty of the waters of the polluted class. It should be noted that they may very probably have been present also in some of the other samples. The primary object of the analysis was to find *B. coli*; and those colonies were selected for subcultures which were most typical of that organism. Again some of the samples which gave no gas in the preliminary dextrose tube and which were not followed further may have contained streptococci. It seems probable, however, that the two groups of sewage organisms normally occur together, and that when this is the case gas will be formed by the *Bacillus coli* in the first fermentation tube. When both colon bacilli and streptococci are present, the latter apparently overgrows the former during the preliminary incubation. The presence of either one then becomes significant. It is desirable that the occurrence of the streptococcus, as well as the colon bacillus, should be noted in sanitary water analysis. With these two apparently characteristic sewage forms, and with perhaps also the *Bacillus sporogenes*, the bacteriologist finds himself in a better position to draw reliable conclusions as to the antecedents of a water sample than has ever before been the case.

In conclusion, we wish to express our thanks to Prof. W. T. Sedgwick, in whose laboratory this work was carried out, for his sympathy and assistance in its prosecution.

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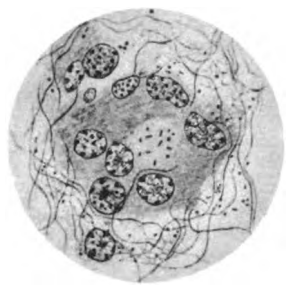
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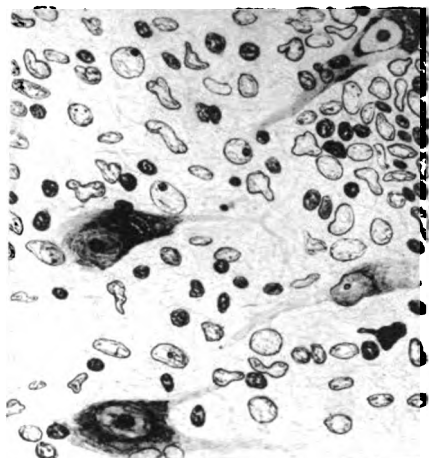


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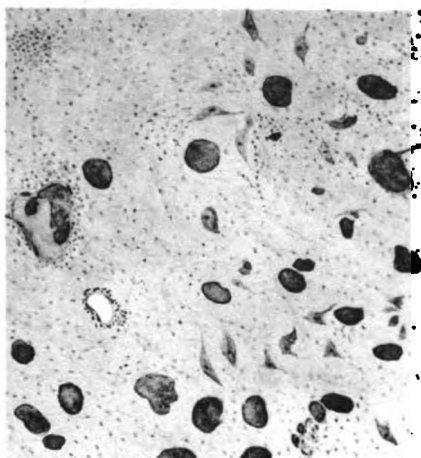
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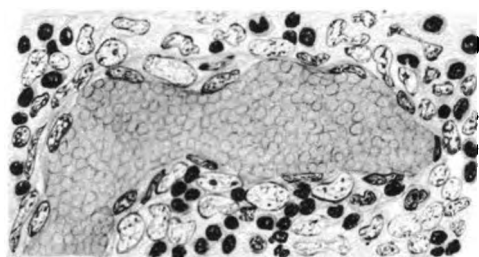
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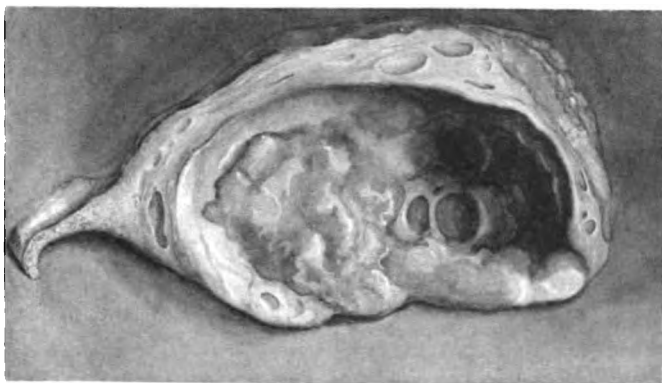
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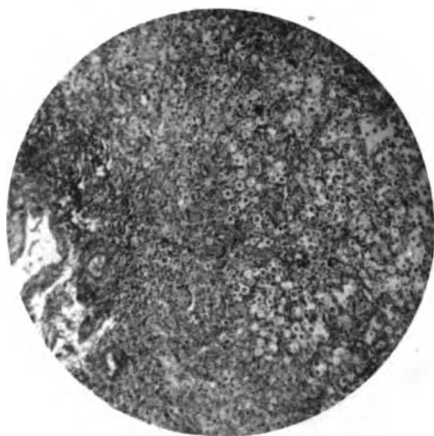
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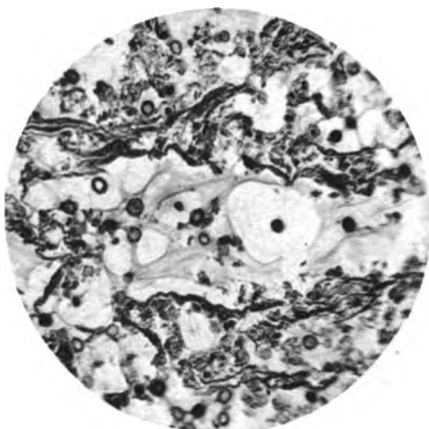
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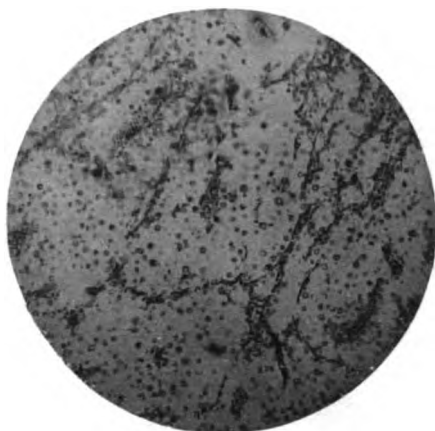
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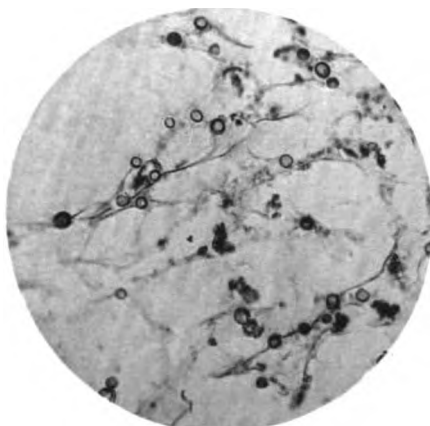
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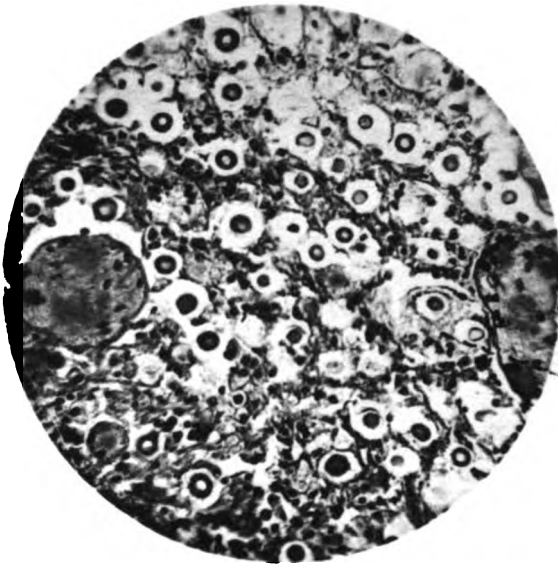
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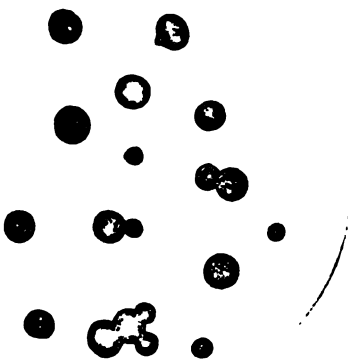
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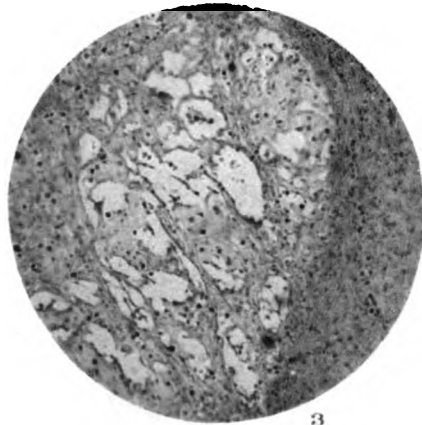
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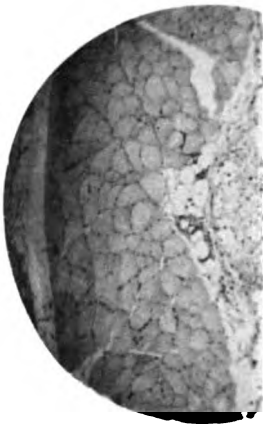
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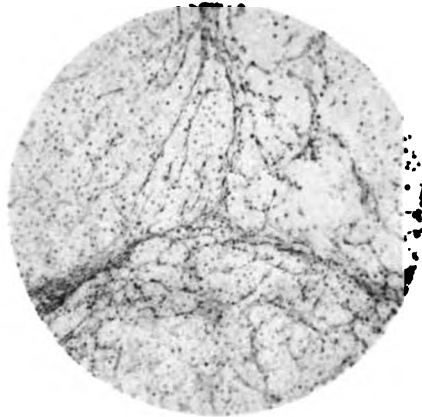
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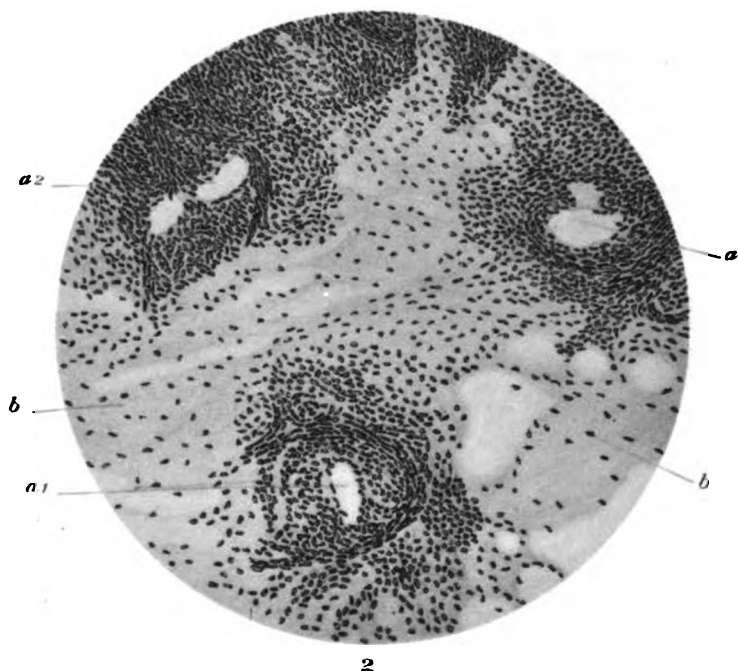
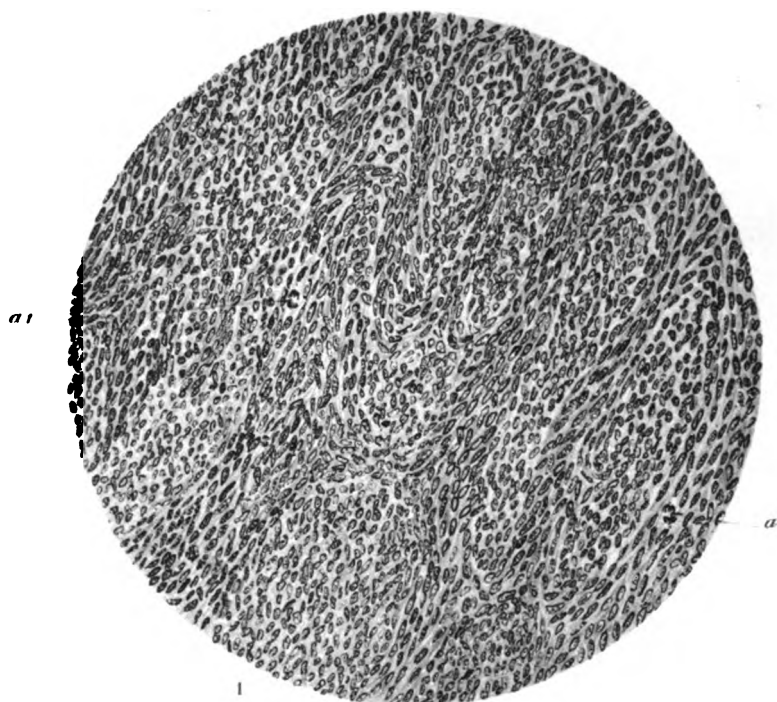
4



5

Frothingham.

Blastomycosis in horse.





1



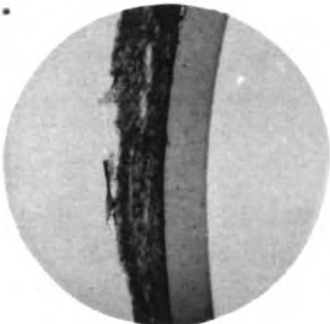
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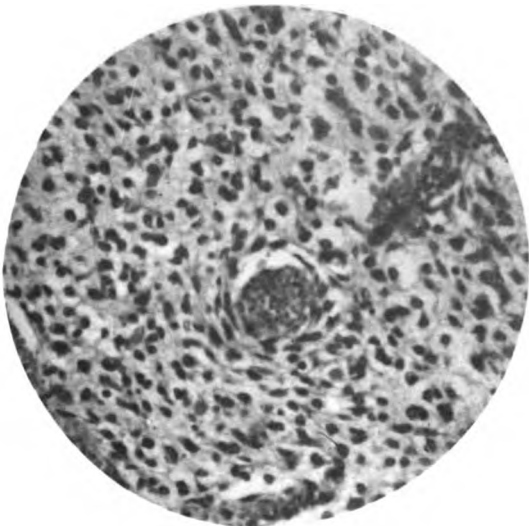
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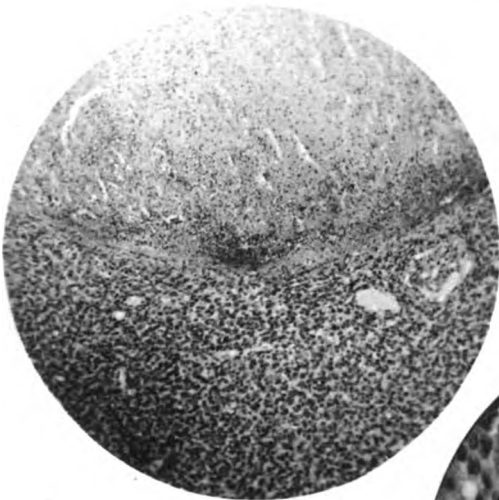
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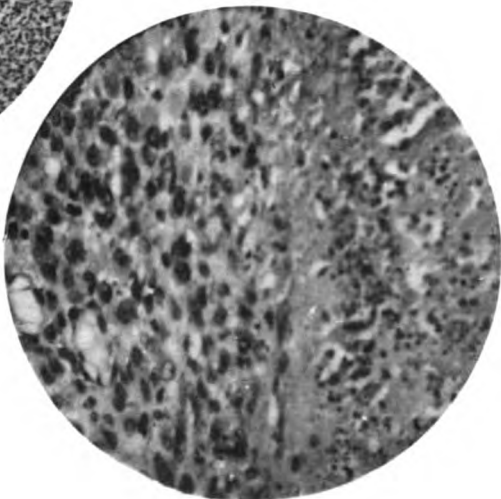
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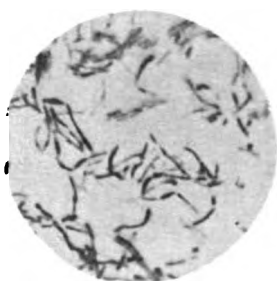
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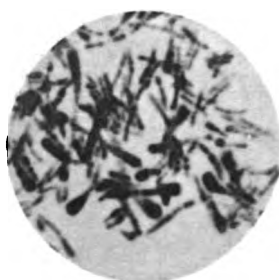
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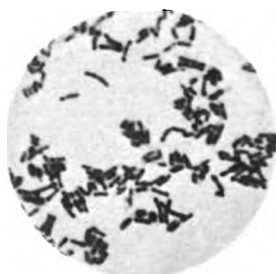
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1



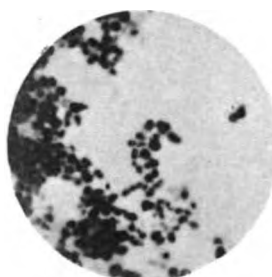
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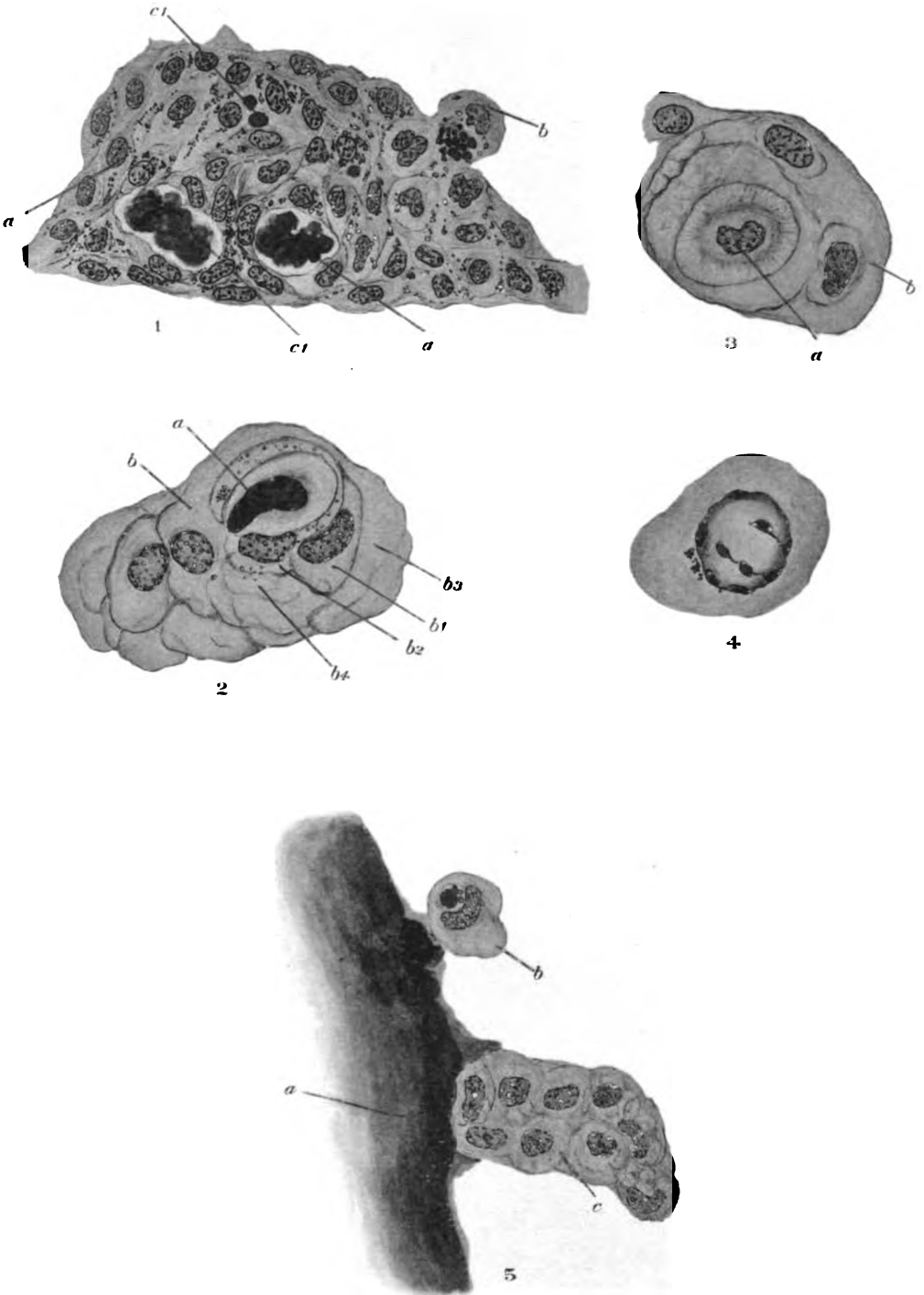
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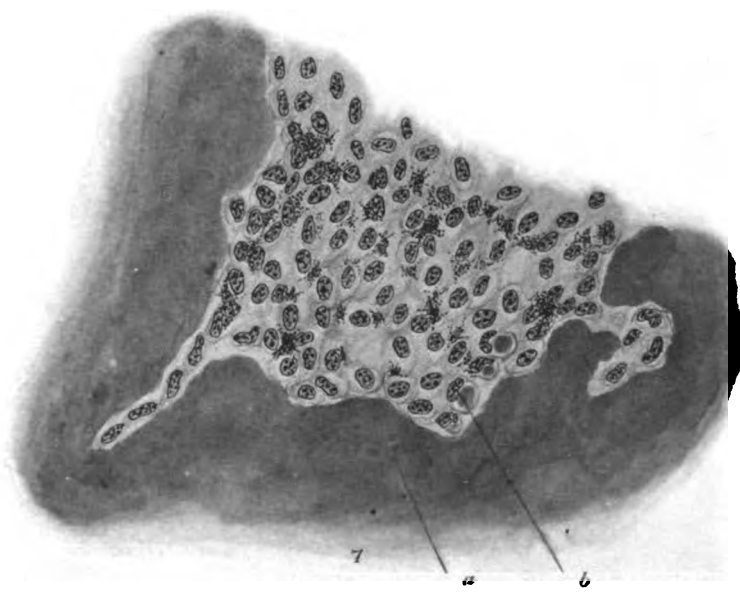
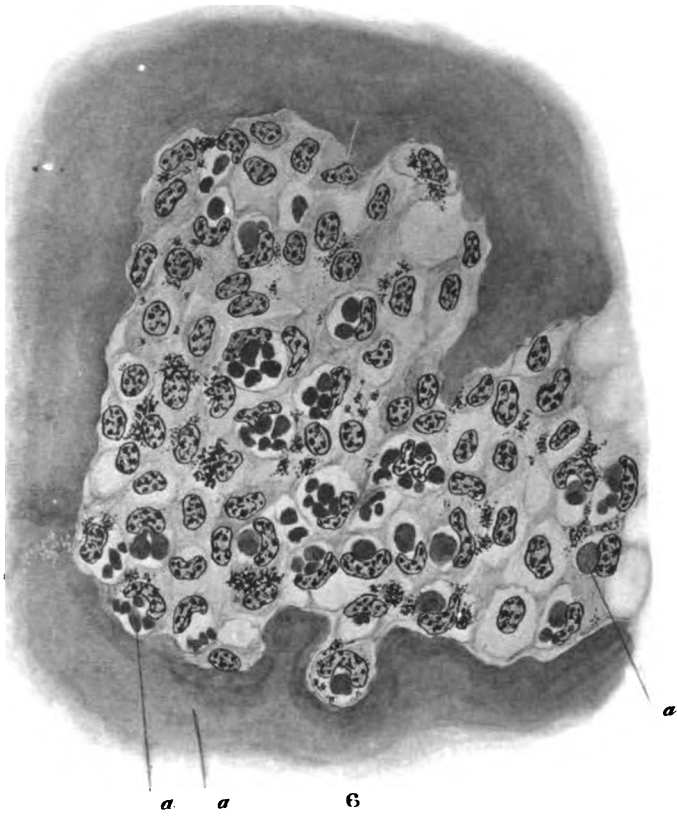
Anna W. Williams.

B. Diphtheriae.



L. Loeb.

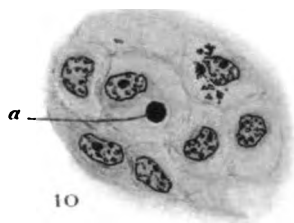
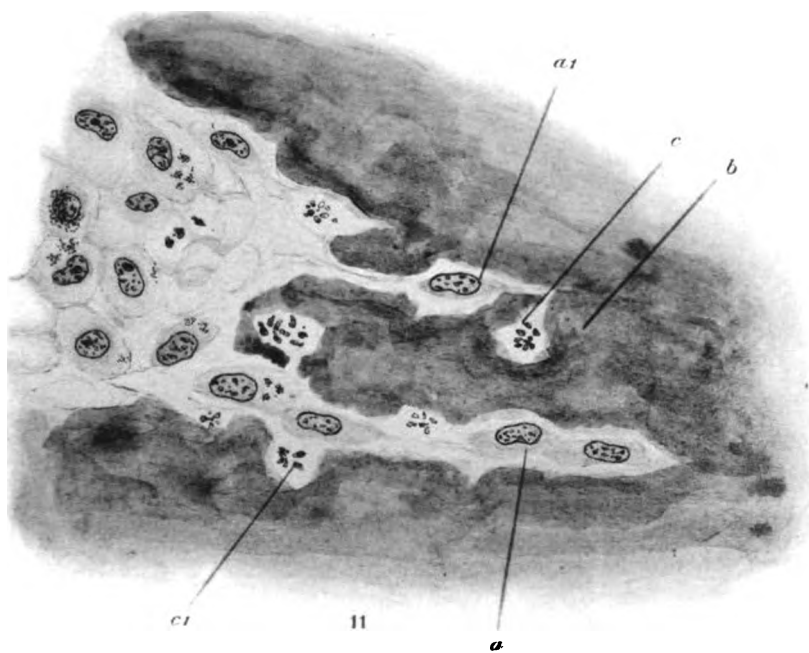
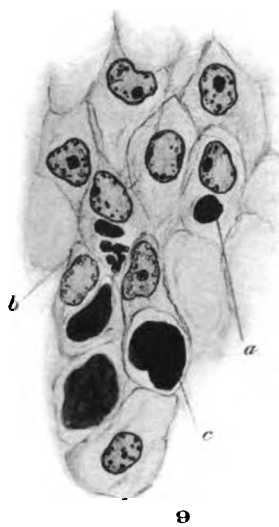
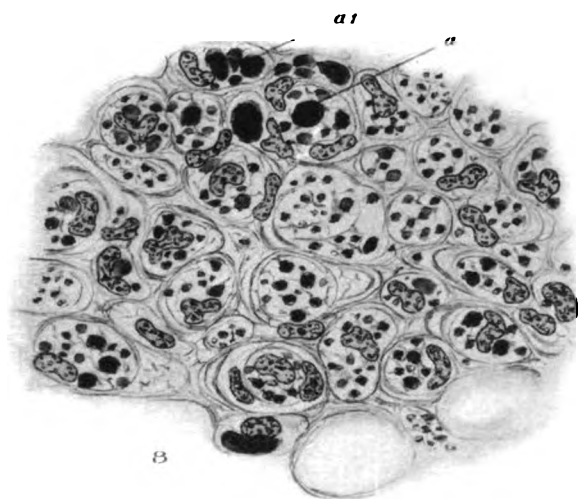
Epithelial growth.



L. Loeb.

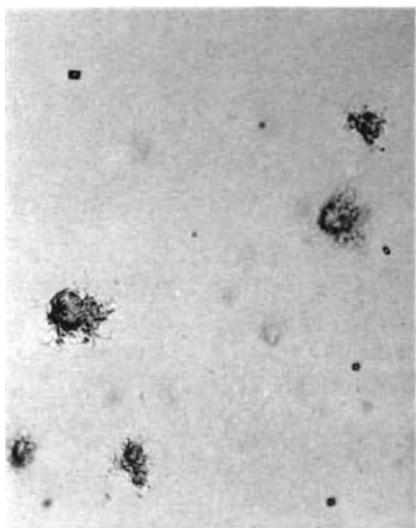
Epithelial growth.

Reproduced from

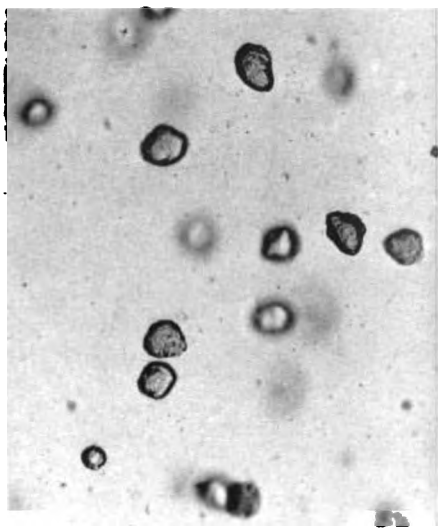


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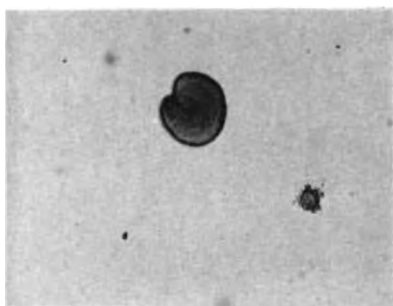
Epithelial growth.



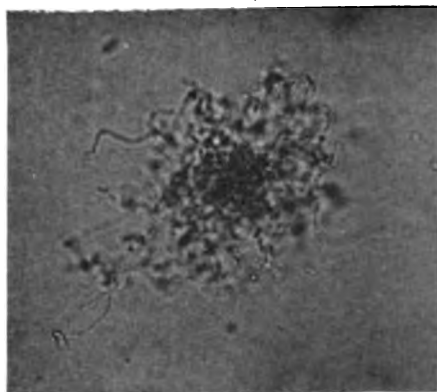
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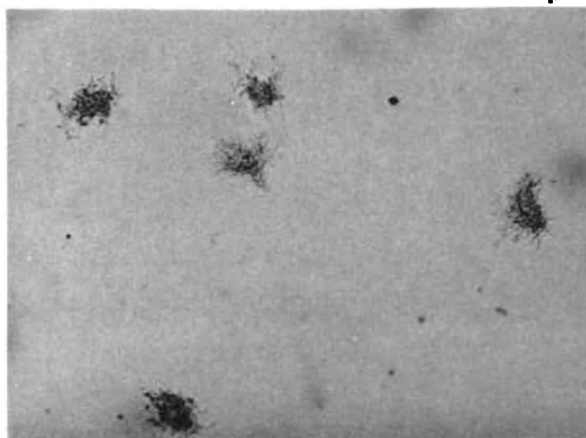
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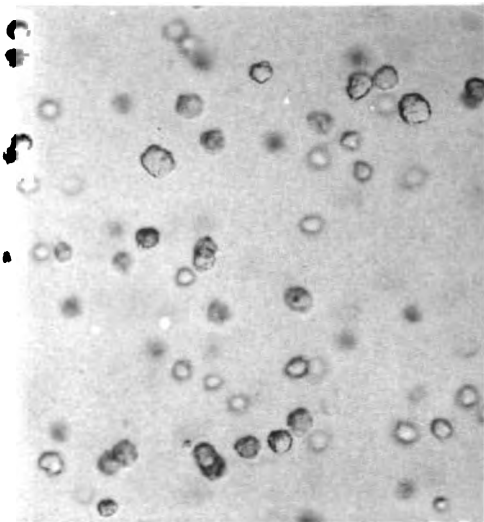


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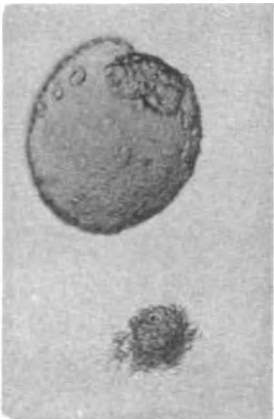
Miss.

Typhoid differentiation

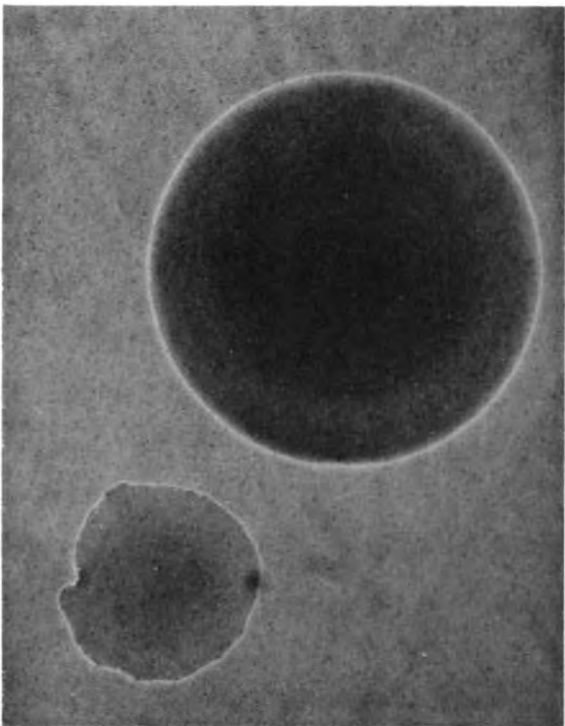
Reynolds, B. and



6



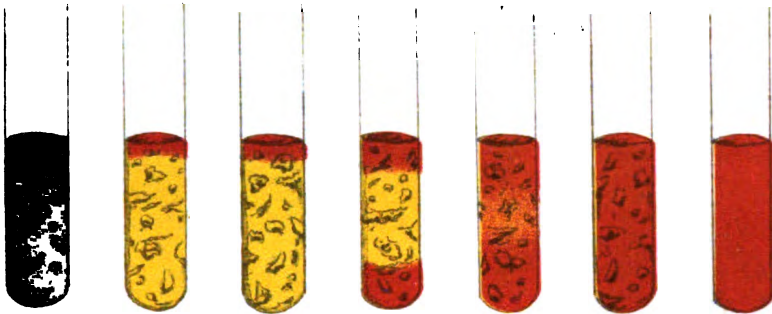
7



8

NEUTRAL-RED AGAR

ONE
DAY



TWO
DAYS



TEN
DAYS



TWENTY
DAYS



B.COLI COMMUNIS

HOG CHOLERA

PARA-COLON GWYN

TYPHOID

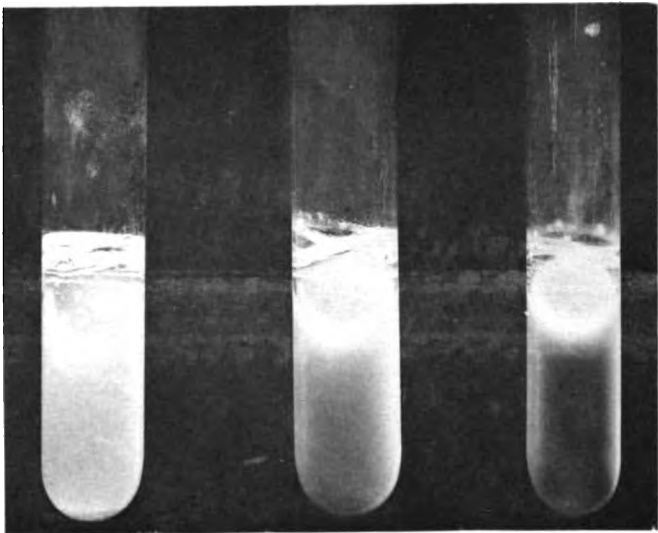
GAERTNER B. ENTERITIDIS

BAC.O.CUSHING

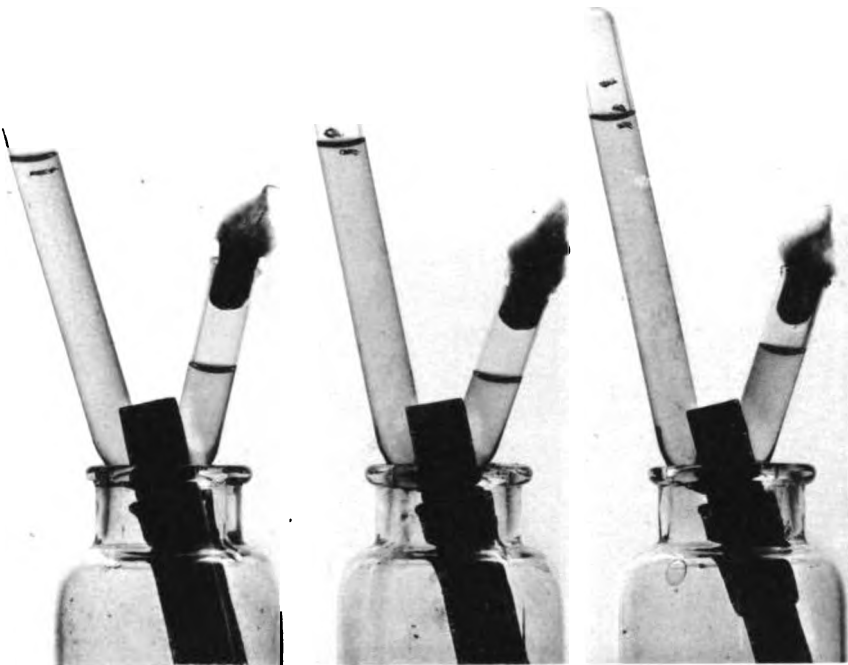
CASE 7

BUXTON.

PARACOLON GROUP.



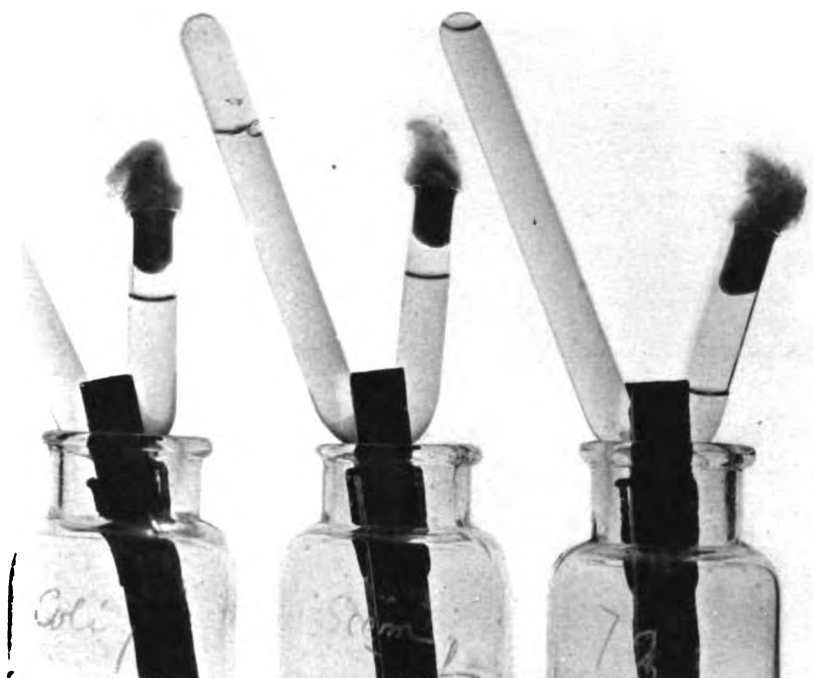
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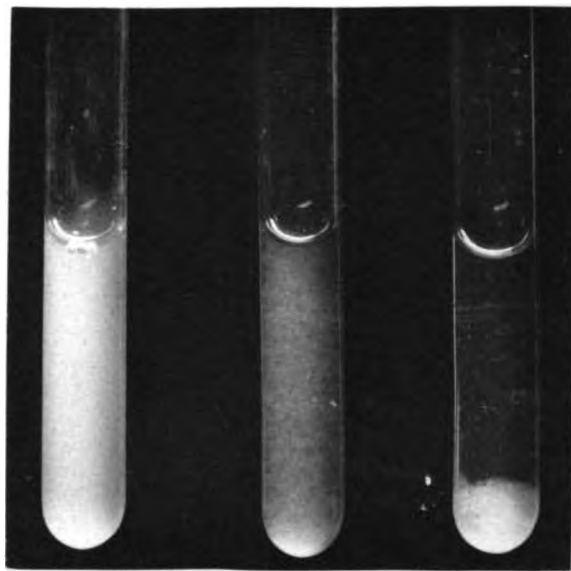
2

Buxton.

Paracolon Bacilli.



3



4

Buxton.

Paracolon Bacilli.

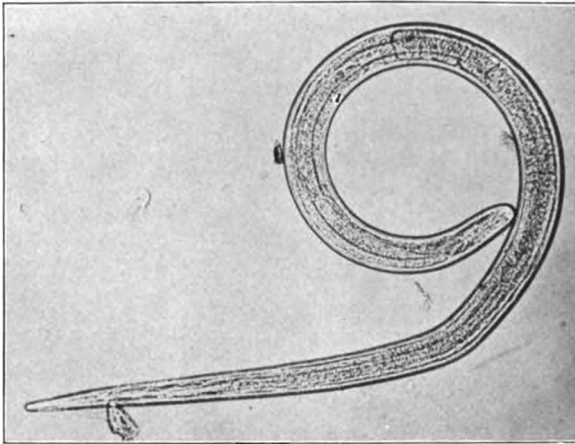


FIG. 1.

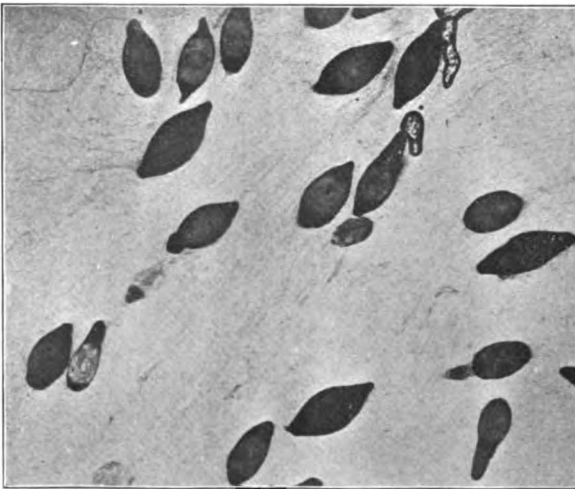


FIG. 2.

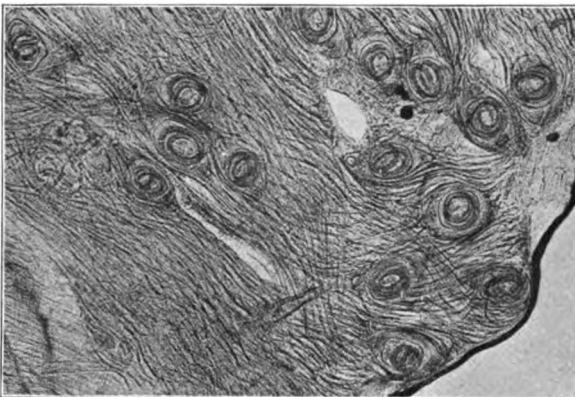
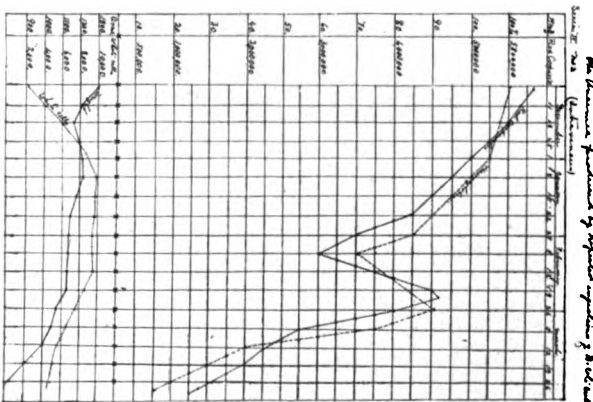
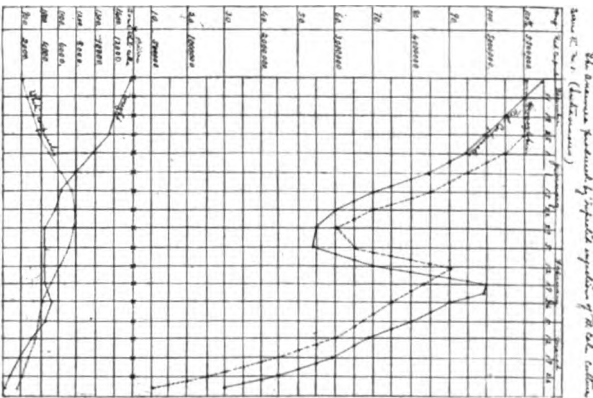
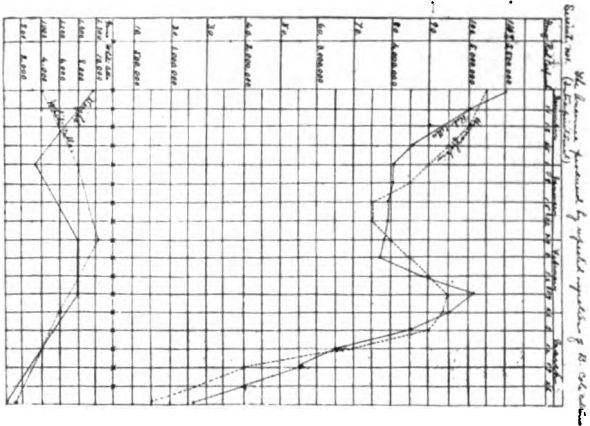


FIG. 3.

DRAKE.

TRICHINOSIS.



CHARLTON.

ANEMIA FROM B. COLI COMMUNIS.

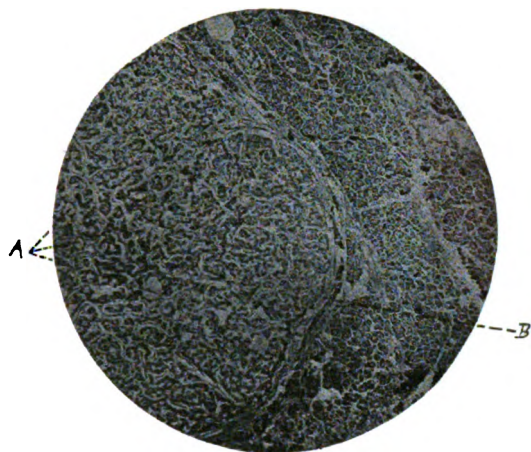


FIG. I. (No. iii. Winckel obj., without eye-piece.)
 Tumor to the left; normal pancreas to the right.
A. Blood extravasations. **B.** Fibrous capsule.

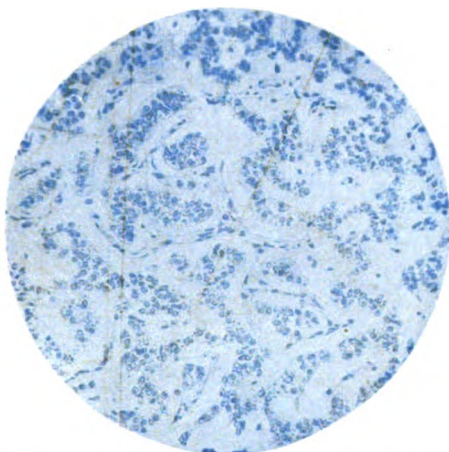


FIG. II. (No. vii. Reichert obj., without eye-piece.)
 Taken from near the center of the tumor. Shows well the thick fibrous septa and the glandular cell-masses. Stained with thionin-blue.

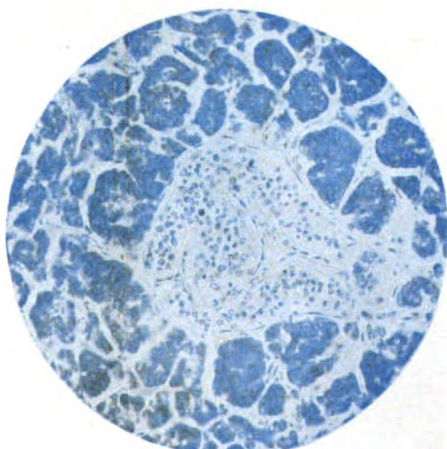


FIG. III. (No. vii. Reichert obj., without eye-piece.)
 An "island" of Langerhans for comparison with Fig. II. The resemblance is close.
NICHOLLS.

ADENOMA OF THE PANCREAS.



1



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9

BUSCH AND VAN BERGEN.

DOG'S BLOOD.

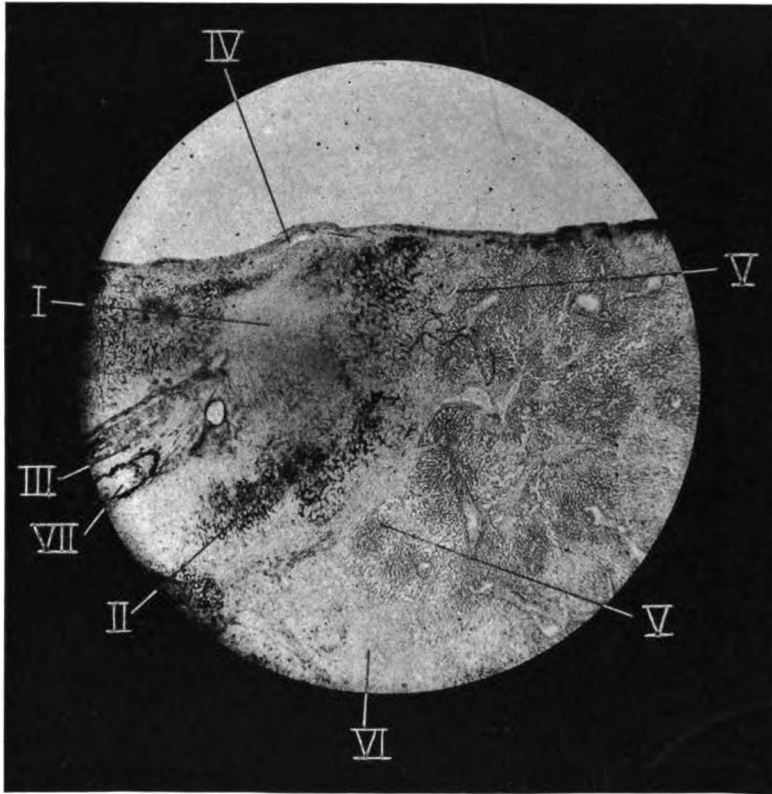


FIGURE I.—Anemic Infarct of Liver.

- I. Central zone of necrosis.
- II. Liver rods at the boundary between the necrosed and living areas, which retain the gentian violet of the fibrin stain.
- III. Glisson's capsule containing necrosed artery (VII.).
- IV. External Glisson's capsule with narrow zone of living tissue.
- V. Boundary zone containing congested living liver rods.
- VI. Area of beginning regeneration.

BALDWIN.

ANEMIC INFARCT OF LIVER.

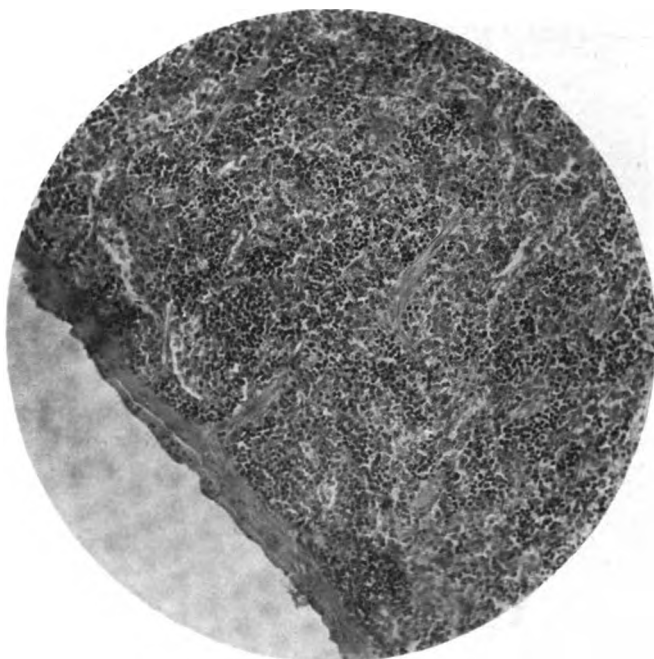


FIG. 1.

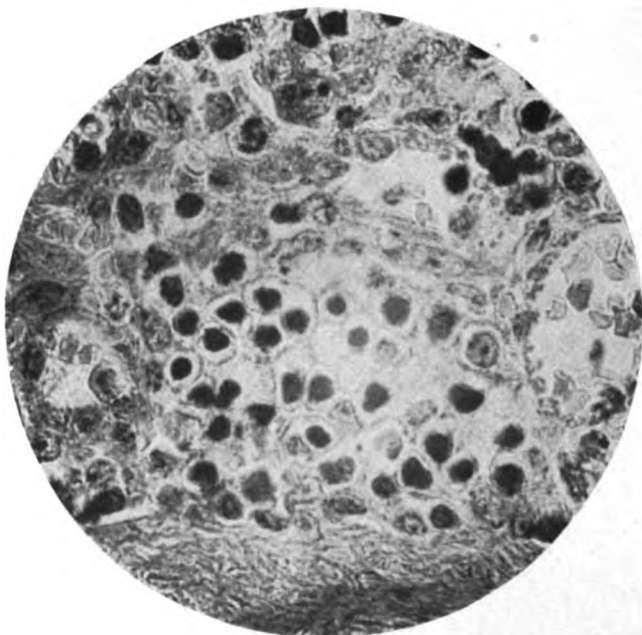


FIG. 2.

BRINCKERHOFF.

ERYTHROBLASTS.



FIG. 1.



FIG. 2.

FLINT.

THE USE OF CLAY MODELS.



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